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TABLE OF CONTENTS

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LOW FIBRINOLYTIC ACTIVITY IN THE WALLS OF VEINS IN PATIENTS WITH THROMBOSIS

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Abst act The fibrinolytic activity of biopsy specimens of veins from 58 patients with superficial thrombophlebitis and/or deep venous thrombosis of the limbs from nine healthy volunteers and from ten patients about to undergo biliary surgery has been stud ed by a modification of the fibrinolysis autography technique of Todd The findings appeared to warrant the following conclusions

1 The f brinolytic activity of unaffected veins in the legs of patients with thrombosis and thrombophlebitis is significantly lower than that of veins in normals

2 In veins occluded by compact thrombi the fibrinolytic activity is negligible but reappears when the thrombi become canalized

It appears that low fibrinolytic activity of the vess l walls predisposes to thrombosis

Using the so called fibrinolysis autography technique" Todd (6) showed that the walls of blood vessels contain a fibrinolytic enzyme the activator of plasminogen It is believed that this enzyme is important in dissolving intravascular clots (1) In postmortem studies Todd (7) rarely found fibrinolytic activity in areas where the thrombi were attached to the vessel walls but often in those where the thrombi had become detached In experimental venous thrombosis in rats Kwaan and Astrup (3) demonstrated that cells from the vascular endothelium are involved in the resolution and canalization of thrombi We recently observed (4) that the concentration of the plasminogen activator in the veins tended to be low in patients with thrombosis a finding suggesting a relationship between low vascular fibrinolysis and the occurrence of thrombosis

The present investigation concerns the localisation and estimation of the fibrinolytic activity in biopsy specimens of veins from a large number of patients with thrombosis of the limbs

MATERIAL AND METHOD

The material consisted of 151 b opsy specimens of veins from 58 patients being followed up because of superficial thrombophlebitis and/or deep venous thrombosis of the arm and/or leg S xteen specimens from ten ambulant patients about to undergo biliary surgery and 27 specimens from nine healthy subjects served as controls The material inc uded the six thrombotic patients and the e ght volunteers reported previously (4) The diagnoses of superficial thrombophlebitis and deep venous thrombosis were based on the classical clinical symptoms the diagnosis of deep venous thrombosis was verified by phlebography in 23 of 33 patients As a rule the biopsy specimens were obtained under local anaesthesia from the great saphenous vein at the level of the medial malleolus and from a superficial vein of the forearm when palpation revealed occluded veins additional specimens were excised from the ipsilateral great saphenous vein but at hi her levels and from apparently normal veins in the saphenous area. The material examined is summarized in Table I.

The veins were examined by a modification of the fibrinolysis autography technique of Todd (6) As known th s method cons sts in incubating sections of tissue in contact with a thin film of f brin rich in plasminogen During the incubation fibrinolysis occurs at the acti e sites of the section and on subsequent stain n, these sites appear as white empty areas surrounded by undigested f brin The method has now been elaborated to permit sem quantitative measurement of the f brinolytic activ ty of the sections and thereby al o compar son of the activity of different samples The strength of the f brinolytic acti ity of the veins was estimated in the following way Four fibrin slides were prepared for each specimen and incubated at 37 C for 0 5 10 20 min respectively Three fairly distinct degrees of f brin di es tion were recognised, namely *g ade I* m croscopical punctate areas of lysis in most of the sections *grade II* gross lytic areas of irregular outl ne and sometimes confluent *g ade III* dissolution of most or all the fibrin in contact with the sections A grade I sl de was allotted 1 point a grade II slide 2 po nts and a grade III slide 3 points. The total number of points scored by the set of four slides was taken as a measure of the f brinolytic

Table I *Biopsy specimens studied*

	No of subjects	Right arm	Left arm	Right leg	Left leg
Patients with thrombophlebitis	25	10	7	33	16
Patients with thrombosis	17	6	7	12	13
Patients with thrombosis and thrombophlebitis	16	12	7	13	15
Controls	19	12	10	11	10

activity cf the samp e Both the method and the reagents u ed are described in detail elsewhere (5) The rank-sum test of Wilcoxon wa u ed to compare the strength of the fibrinolytic activities of different groups of veins

RESULTS

Controls

All the veins were microscopically normal Most of the fibrinolytic activity was confined to the vasa vasorum of the adventitia The media con tained a smaller number of lytic areas while the intima was only rarely active Arm veins were more active than leg veins (Table II)

Thrombosis and Thrombophlebitis

e biopsy specimens were too small to allow ultaneous processing by standard histological chniques The microscopic evaluation of the pathological material was therefore somewhat crude It was however possible to separate these

Table II *Fibrinolytic activity in arbitrary units of limb veins from healthy subjects and patients with thrombosis and/or thrombophlebitis*

Figures within parentheses denote the number of specimens

	Patent veins	Veins occluded by compact thrombi	Veins containing canalized thrombi
Arms			
Controls	7 73±1 81[a] 2 5-11[b] (2?)	(0)	(0)
Patients	6 09±2 21 0-9[b] (48)	1[a] (1)	(0)
Legs			
Controls	4 69±2 11[a] 1-8 5[b] (21)	(0)	(0)
Patients	2 53±2 37[a] 0-8 5[b] (61)	0 46±0 09[a] 0-3[b] (25)	3 91±2 71[a] 0-8[b] (16)

[a] Mean ±s.d [b] Range

veins into two main groups one with patent vein and one with thrombosed veins

Patent veins

In the patent veins of normal appearance th intramural distribution of the fibrinolytic activit was the same as in those from subjects withou thrombosis An illustrative example of this pat tern of activity is given in Fig 1 As in th controls arm veins were more active than le veins (Table II) The mean fibrinolytic activity o patent arm and leg veins of patients with throm



Fig 1 Unaffected leg ve of a patient with thrombosi Numerous lytic areas relat to the adventitia and the media Incubation time 1(min Magn ×12



Fig 2 Saphena magna filled by a compact thrombus In spite of the prolonged incubation time (0 min) there is no fibrin digestion The arrow points to an inactive vasum vasi in the adventitia Magn. ×20

bosis was lower than that of the veins of the controls The difference was highly significant for the veins of the leg ($P < 0.001$) but not significant for the veins of the arm ($P = 0.06$) Of the 61 patent leg veins from the thrombotic patients 15 (about 25%) were completely inactive

Thrombosed veins

This group was also divided according to the presence or absence of canalizing vessels in the thrombus

Veins occluded by compact thrombi The fibrinolytic activity of these veins was negligible (Table II) Only one arm vein was occluded Its activity was 1 Of the 25 specimens from the leg as many as 19 had no demonstrable fibrinolytic activity A specimen of a thrombosed vessel showing no activity even after prolonged incubation is shown in Fig 2 In the few thrombosed veins in which activity was demonstrated it was confined to the vasa vasorum in the adventitia and media The thrombi themselves were inactive although lysis due to disrupted cells presumably leucocytes was occasionally observed

Veins containing canalized thrombi On canalization of the thrombus fibrinolytic activity re



Fig 3 Occluded saphena magna A single area of fibrinolysis is produced by a newly formed vessel sprouting from the intima. The other newly formed vessels are inactive Incubation time 10 min Magn. ×30



Fig 4 Saphena magna containing a canalized thrombus. Numerous lytic areas related to the canalizing vessels Incubation time 10 min Magn ×20

appeared and was then stronger than in patent veins (Table II) The difference however was not significant ($P = 0.07$) Canalization began from active capillary buds some of which could be seen growing from the intima (Fig 3) Canalized vessels usually possessed high fibrinolytic activity (Figs 4 and 5) but in some cases inactive canalizing vessels were observed

DISCUSSION

The present observation that the walls of veins occluded by thrombi contain practically no fibrinolytic activators strengthens the belief that these enzymes are important in dissolving intravascular clots The lack of activity of thrombosed veins may tentatively be explained by the assumption that the pre-occlusion plasminogen activator in the vessel has been consumed by attempted physiological thrombolysis or that the pre occlusive fibrinolytic activity of the vein walls was low and facilitated the development of the thrombus The latter possibility is supported by the finding of abnormally low fibrinolytic activity in unaffected veins from patients with thrombosis and secondly by the observation that the fibrinolytic activity of the blood after venous stasis is substantially lower in patients with

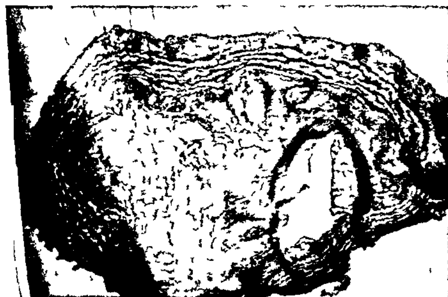


Fig 5 Major canalizing vessel producing a large area of fibrinolysis Incubation time 5 min. Magn ×18

thrombosis than in controls (2 4) As known stasis is believed to liberate fibrinolytic activators from the vessels (1) It seems justified to consider the possibility of a decreased content of fibrinolytic activators of the vessel walls as a contributory factor in the causation of thrombosis Whether the decreased fibrinolytic activity of the veins of thrombotic patients is a primary disorder or secondary to some other disturbance must await further research

The activity observed in the canalizing vessels is in agreement with the findings of Todd in autopsy material (7) and of Kwaan and Astrup in the rat (3)

ACKNOWLEDGEMENTS

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CYTOMEGALOVIRUS INFECTION AMONG RENAL ALLOGRAFT RECIPIENTS

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Abstract A comprehensive serologic virologic and histologic study has been made of the incidence and clinical significance of cytomegalovirus infection among 43 renal allograft recipients. Material adequate for investigation was available from 36 patients and evidence of infection was found in 33 giving an incidence of 91 In the majority of patients infection occurred during the first two months after transplantation Most infections were clinically inapparent, but in four patients a febrile illness associated with a relative lymphocytosis was seen which it seems reasonable to assume was caused by infection with cytomegalovirus Cytomegalovirus pneumonia based on the finding of cytomegalic cells in the lungs was seen in 7 of the 17 autopsied patients. Although there was extensive cytomegalovirus pneumonia in three patients death was not considered to have been caused by or indeed significantly contributed to the infection, as all had a bacterial pneumonia as well

Since 1963 the frequent occurrence of cytomegalovirus (CMV) infection among kidney transplant recipients has been well documented in several reports from two transplantation centers in the United States Boston and Denver A characteristic feature of CMV infection is the presence of large cells with hypertrophied nuclei containing intranuclear inclusions the so-called cytomegalic cells in tissue infected by the virus and the early studies of CMV infection were based on autopsy findings Hill et al (6) in 1964 mentioned the high incidence of CMV infection in connection with a general discussion of opportunistic infections observed in patients treated with immunosuppressive and cytolytic agents Hedley Whyte and Craighead (5) confirmed this finding and in addition they were able to isolate CMV from the lungs of a patient with severe pneumonia in which cytomegalic cells had been found on histologic examination In 1965 Rifkind (12) reported virologic studies in three patients with pulmonary cytomegalic cells and noted in addition, an increase in CMV complement fixing (CF) antibodies in kidney transplant recipients Kanich and Craighead (7) found that it was often possible to isolate CMV from the urine and oropharyngeal secretions of patients after transplantation These investigators have shown that CMV infection is frequently generalized as the virus may be isolated from several organs at autopsy Cytomegalic cells have also been found in many parts of the body including the central nervous system (14) but these cells are unusual in organs other than the lungs in kidney transplant recipients

An analysis of the findings from both American centers was published in 1967 Among 51 autopsied cases Rifkind et al (13) found cytomegalic cells in 52% while cytomegaloviruria was demonstrated in 17 of 26 living recipients Three patients had clinical symptoms of pneumonia in association with a rise in CMV CF antibody In all of the autopsied patients with pneumonia in whom cytomegalic cells were found in the lungs other infectious agents usually bacteria, were always present. Craighead et al (3) studied 41 renal allograft recipients using serologic and virologic techniques and found evidence of CMV infection in 73% of patients surviving one or more months after transplantation The occurrence of the infection did not correlate with a well-defined clinical syndrome even though generalized CMV infection was found in some patients at autopsy These investigators also mention that infection may be either primary or the result of reactivation of latent infection

Finding on autopsy of cytomegalic cells in the lungs and renal graft of one of the first patients in the Aarhus series (10) stimulated our interest in this problem The object of this investigation has been to evaluate the extent and significance of CMV infection among recipients of a renal allograft in the Aarhus series

The studies which will be reported here began in November 1966 and were prospective as well as retrospective

MATERIAL AND METHODS

Originally only a prospective investigation was planned All patients transplanted in the fall of 1966 and thereafter were studied serologically and virologically at monthly intervals for the presence of CMV infection Later it proved possible to include several patients transplanted earlier This retrospective study included both histologic and serologic investigations.

Patient material

Forty three patients, 21 women and 22 men, with an average age of 31 years have received renal transplants at this Center from April 1964 to the 15th of February 1968 Twenty of these patients have died as of May 1968

Immunosuppressive therapy

Azathioprine 2-4 mg kg body weight, was used from or before the day of transplantation in all cases. Prednisone 300 to 150 mg day was started at the first sign of a rejection crisis and thereafter slowly reduced to a maintenance dose of 10 to 20 mg day unless renewed signs of rejection appeared whereupon the dose was again increased. During the period covered by the prospective study November 1966 to May 1968 the average daily dose of prednisone given has been 1 43 mg kg body weight during the first post transplant month and 0 61 mg kg and 0 37 mg kg during the second and third month. Actinomycin C, 200 μg day was given at the time of a rejection crisis in some patients and was in some cases supplemented with 150 r X irradiation.

Virus isolation

Human embryonic fibroblasts from muscle (HEM) or lung (HEL) were used for virus isolation and the production of virus antigen. Cell cultures were prepared from embryonic tissues after treatment with a solution of 0.05 % trypsin and grown in one liter Roux Flasks. Tissue cultures were used from passages 3 to 30 Eagle s basal medium (EBM) supplemented with 10 calf serum containing 200 IU penicillin and 100 μg streptomycin per milliliter was used as growth medium, while EBM with 2% calf serum and antibiotics served as maintenance medium. 100 μg kanamycin and 25 μg mycostatin per milliliter were added to cell cultures employed in virus isolation to prevent the growth of unwanted microorganisms. Culture medium was changed three times a week. Cell cultures in roller tubes or 200 ml flasks were used for virus isolation. All cultures were incubated at 37 C Oropharyngeal washings, using culture medium without antibiotic urine and blood were cultured for virus. The technical procedure employed was modified during the study Originally 0.3 ml urine or throat washing was inoculated onto culture tubes after storage for one hour at 4 C later only freshly voided urine was either added directly to cell cultures (1 ml per flask) or mixed with equal parts EBM containing 0 2% bovine serum albumin at 37 C and the pH adjusted to 7 0 with a 2 8 % solution of sodium bicarbonate Two ml of this mixture was added to a culture flask and 40 to 50 ml of the rest was centrifuged at 60 000 g for two hours in a Spinco L-2 ultracentrifuge using Rotor L 50 Nine tenths of the resultant supernatant was discarded and the rest, together with the sediment, was inoculated onto a flask culture at times the same as that to which freshly voided urine had been added. Blood was cultured for CMV by placing 10 ml fresh heparin-stabilized blood in an incubator at 37 C for one hour allowing the red blood cells to settle out. The plasma was then centrifuged at 1000 g for 15 min, the supernatant discarded and the sediment added to a culture flask after suspension in a few drops of phosphate buffered saline (PBS) Autopsy tissue was ground in a mortar with sand and PBS added to make a 10% suspension. After centrifuging at 1500 g for 15 min one ml of the supernatant was placed in a culture flask Culture medium was changed 3 to 5 hours after inoculation

Virus identification

Tissue cultures were examined at weekly intervals for cytopathic effect for 6 to 8 weeks. Cultures not demonstrating cytopathic effect were regarded as negative and discarded Positive cultures were trypsinized with a 0 1 % trypsin solution after cytopathic effect had developed sufficiently One third of the cells were transferred to a new culture flask, another third were placed in a Petri dish containing three cover glass cultures, and the rest were frozen at −70 C Cover-glass cultures were incubated in an airtight box under 5 CO₂ On the appearance of cytopathic effect, one cover glass culture was fixed with Bouin s solution stained with hematoxylin-eosin and examined microscopically for the presence of nuclear and cytoplasmic inclusions The two other cultures were used in an immunofluorescence study using Coons indirect technique One known negative and one known positive convalescent serum together with the patients own serum were employed as the primary sera to different parts of the cover glass cultures, and a fluorescein-conjugated antihuman γ-globulin produced in rabbits served as the secondary serum. All sera were diluted 1 10

In some cases a CF antigen was prepared from a flask culture after the first passage in order to investigate the antigenic characteristics of the newly isolated strain.

Serological tests

Antigen was prepared by inoculating 1 liter Roux flasks containing human embryonic fibroblasts with 2 5×10^4 PFU (plaque forming units) of CMV strain AD 169 (the

strain supplied by Dr Gun Carlstrom, Karolinska Sjukhuset, Stockholm) After the development of diffuse cytopathic effect, which took 1 to 14 days, the medium was decanted and 5 ml sterile water added Cells were shaken or scraped free and the cell suspension freeze-thawed twice Cell debris was removed by centrifugation and the antigen containing supernatant stored in sealed glass ampules at −70 C until used.

A standard microtiter CF test (Cook Engineering Co) was used employing 2 units of antigen, 1 / units of complement and 4 units of hemolysin Antigen, serum and complement were incubated for 18 hours at 4 C. After warming to 37 C the hemolysin system was added and the specimen incubated for 30 min at 37 C 25% hemolysis served as end point on titration All sera were first tested in a 1 4 and 1 8 dilution against both CMV antigen and an antigen prepared from a non infected cell culture according to the method described above Sera were also tested for anticomplementary activity Positive sera were then titrated against CMV antigen in doubling dilutions from 1 2 to 1 256 together with a serum with known titer All sera from each patient were parallel titrated upon completion of the follow-up period.

Sera from patients in the prospective study were primarily tested after 1 to 3 days at 4 C and then stored at −20 C for later parallel titration. Sera from most of the patients in the retrospective study had been stored for 1 month to 4 years at −20 C a few sera, however had been preserved by lyophilization. All sera were inactivated at 56 C for 30 min.

In studying neutralizing (NT) antibody the plaque reduction test of Plummer and Benyesh-Melnick was used (11) 7.5×10^5 cells suspended in growth medium were grown to confluence in plastic Petri dishes, 6 cm in diameter in a humified 5% CO incubator Serum diluted 1 2, 1 10 1 50 and 1 250 with TRIS-buffer was mixed with a cell free virus suspension of strain AD 169 and incubated for 1 hour in a waterbath at 37 C After replacement of the culture medium by 0 3 ml TRIS-buffer 0.2 ml from each dilution of the serum and virus mixture was inoculated onto two dishes and the cultures again placed at 37 C for one hour with tilting of the dishes every 15 min Thereafter the serum and virus mixture was poured off and the cultures covered by a solid layer of EBM with 10% calf serum, 2% methyl cellulose sodium bicarbonate and antibiotics, and again incubated for 14 days. Once or twice during that period a new layer of the above mentioned overlay was added At the end of the 14 days the dishes were placed at 4 C for a few hours, the medium removed and the dishes stained overnight with 0.3% methylene blue The resultant plaques were then counted with the aid of a diascope The titer of the virus suspensions was regulated on the basis of previously made serial titrations so that the virus suspension yield d 30 to 90 plaques with a negative serum and the accuracy and reliability of the test were controlled at each titration by employing known positive and negative sera. Serum dilutions showing 80% plaque reduction were used as the end point. Titration of virus stocks was also performed by the plaque method.

Histological studies

Lung sections (c 2×2 cm) from the autopsied cases, generally three from each patient, were carefully examined for the presence of cytomegalic cells after fixation in 4% neutral formalin and staining in hematoxylin-eosin.

RESULTS

The following criteria were used in this study in diagnosing CMV infection

1 Positive sero-conversion or a fourfold rise of CF antibody on parallel titration.
2 Significant rise in NT antibody
3 Isolation of virus
4 Finding of cytomegalic cells in lung sections on autopsy

Serological studies

For serological determinations c 450 sera were available from 34 of the 43 patients transplanted in the study period.

It was not always possible particularly in the retrospective group to obtain sufficiently representative material Data on the patients studied the material available and results achieved are shown in Table I No sera at all were available from one patient (in the retrospective study) and from eight others only sera obtained near the time of transplantation could be located Seven of these died soon after transplantation two in the first four in the second and one in the third post transplant month Paired sera from the eighth patient who is still alive were first available from post transplant day 200

At least a fourfold increase in CF antibody or seroconversion was demonstrated in 29 but there was a serious lack of sera from the days immediately after transplantation in one of these patients Details concerning the serologic findings in the remaining 28 patients are given in Tables II and III In ten patients (Table II) no CF antibodies against CMV were detected in the initial sera but in seven of these it was possible to demonstrate a low titer of NT antibodies In 18 patients (Table III) CF antibodies were demonstrable in sera taken before or in a few cases immediately after transplantation There was no relationship between the age of the patients and the presence or absence of CF antibodies as the average age of both groups was 31 years As

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In some cases a CF antigen was prepared from a flask culture after the first passage in order to investigate the antigenic characteristics of the newly isolated strain.

Serological tests

Antigen was prepared by inoculating 1 liter Roux flasks containing human embryonic fibroblasts with $2.5 \times 10^{?}$ PFU (plaque forming units) of CMV strain AD 169 (the

Table III *Results from 18 patients with a fourfold or greater rise in CF antibodies against CMV*

Pat no	Initial CF titer	Complement fixing antibody: Days post transplant between which a four fold or greater rise in titer registered				First serum with highest titer		Latest titer		Living or dead
		Month 1	Month 2	Month 3	Month 4 or later	Titer	Day	Titer	Day	
T 3	8		35–45			64	45	a		D
T 7	32	(−2)–17				256	17	3.	314	D
T 9	4		23–60			64	60	<4	320	D
T 11	16				78–100	128	125	a		D
T 12	32				98–126	256	126	16	956	L
T 15	4			4? 77		1 8	77	64	778	L
T 16	8		21–46			128	64	32	736	L
T 18	4			56–74		256	92			D
T 20	4	(−1)–22				.56	2.	8	515	L
T 23	16				23–198	64	198	64	4 8	L
T 24	8		27–54			256	116	64	418	L
T 26	8			4 –80		64	151	16	456	L
T 27	2			35–76		16	76	<2	448	L
T 30	8	(−13)–28				128	94	128	216	L
T 31	16				108–12.	256	12.	64	205	L
T 33	2		13–47			64	47	a		D
T 42	4			55–83		16	83	16	93	L
T 43	4	23– 6				16	.6	16	40	L

No sera available after antibody rise

A graphic illustration of the serologic studies by the CF and NT tests in six patients is given in Figs 1 and 2 In several sera from these six patients NT antibodies against strain AD 169 were demonstrated These antibodies were found in patients with CF antibody before transplantation and in patients 23 24 and 26 a parallel increase in CF and NT antibodies was seen but not in patient 27

Patients 28 and 29 who did not have d tectable CF antibodies at the time of transplantation did not have NT antibody either A marked rise was however demonstrated in these patients but it was somewhat delayed in relation to the rise in CF antibody as the first sera to show a rise in CF antibody did not contain demonstrable NT antibody In another four kidney transplant patients we have recognized a rise in NT antibody against AD 169 after increase in CF titer

A significant decrease in CF antibody titer against CMV was often registered in the *first sera* after transplantation such as that seen in patients nos 26 (Fig 1) and T II (Table V) A similar phenomenon was seen in som deceased pati nts with d tectable CF antibody before

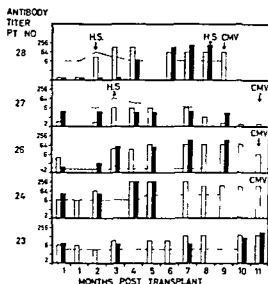


Fig 1 Stud es of cytomegalovirus complement fixing and irus neutralizing antibodies in five patients after kidney transplantation. White columns represent titer of comple ment fixing and black on s virus neutralizin antibodies a ainst cytome alo irus The broken line depicts the titer of herpes simplex virus complement fixin antibody Isolation of cytomegalo iru (CMV) and herpes s mplex virus (HS)

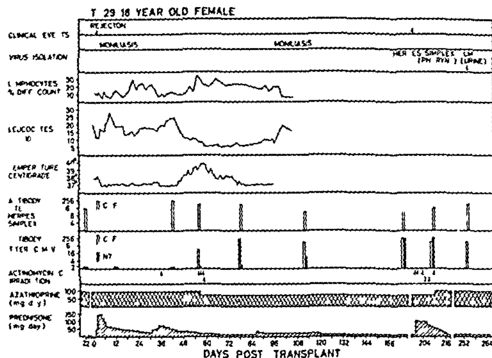


Fig 2 Post transplant course of patient T 29 with a febrile illness in association with rise of complement fixing and virus neutralizing antibodies against cytomegalovirus

transplantation and no titer rise in the post transplant sera

Also illustrated in Figs 1 and 2 are the results of an investigation of CF antibody against Herpes simplex virus in the same sera A significant increase was seen in three and Herpes simplex virus could be isolated from throat washings from all three There was no CF antibody to Herpes simplex virus in any of the sera from patient 26 Generally there was no corresponding increase in these antibodies after transplantation similar to that seen with CMV

Virus isolation

Attempts at isolation of CMV from renal graft recipients by inoculating urine throat washings and lung suspensions onto tissue culture may involve a great deal of tedious work The main reason for this is that tissue cultures often become contaminated by microorganisms resistant to the antibiotics usually used in tissue culture media We have attempted to solve this problem by adding to the culture media antibiotics to which the bacteria usually identified as Pseudomonas sp were found to be susceptible according to sensitivity tests In general kanamycin (100 μg/ml) proved sufficient to prevent bacterial contamination Occasionally it was necessary to use chloramphenicol (50 μg/ml) or colimycin B (50 units/ml) in addition in order to carry the cultures until either a viral effect could be observed or the cultures discarded as negative This circumstance and the fact that some of the inocula contained cytotoxic substances resulted in destruction of some or all of the tissue cultures in about half of the attempts at virus isolation

Only the isolation attempts in which cultures were kept alive for at least 30 days or in which viral growth was noted are included in this report Virus isolation has generally not been attempted among patients in the retrospective group

Longitudinal virologic studies were conducted in one group of ten patients for up to 10 months after transplantation and it was possible in these patients to carry out 88 virus isolation attempts by inoculation of urine and throat washings onto roller tubes All 44 urine isolations were negative but Herpes simplex virus was isolated from throat washings six times in five patients

Later flask cultures inoculated with freshly voided urine or ultracentrifuged urine were used

able IV *Results of investigations for CF antigens of isolated strains from kidney transplant recipients*

	Titer	Isolated strains				
a	AD 169	T 33 lung	T 29 urine	T 28 urine	T 36 urine	T 16 urine
egative	<2	<2	<2	<2	<4	<4
egative	<4	<2	<2	<2	<4	4
gative	<4	<2	<	<2	<4	<4
tive	<4	<2	<2	<2	<4	<4
egative	<4	<2	<2	<2	<4	<4
29 day 27	<4	<2	<2			
36 day 9	<2	<2	<2	<2	<4	<4
36 day 57	64				32	32
36 day 79	32				16	16
i 36 day 86	32	32	64	32		
ive	32	32	64	32	3	32
ositive	8	16	8		16	16
itive	16	16	32	32		
ive	128	256	256			
ive	64	256	128			
i e	32				32	32

n 30 isolation attempts from 15 patients CMV as recovered 16 times from 11 patients in this way and what were probably adenoviruses were isolated from three patients during one month in the fall of 1967 We did not isolate CMV from the urine of patients in whom a rise of CF titer had not been demonstrated In one patient virus was isolated 26 days after sero-conversion In another viruria was first demonstrated twice about 300 days after rise of the titer at a time when CF antibodies were no longer detectable in the patient s serum Virus was found in the urine from another patient 717 days after antibody rise

CMV was isolated from the peripheral blood once on 8 attemps from seven patients This patient died on post transplant day 83 without any rise in CF antibody but there was a titer in the initial serum The inoculation of a suspension of lung tissue onto flask cultures from two patients in whom cytomegalic cells had been found on histologic examination of the lungs resulted in the isolation of CMV in both cases

The typical cytopathogenic effect of CMV was generally seen in flask cultures 11–27 days after inoculation with infective material

Virus identification

The strains of CMV isolated in this study possessed characteristics similar to those of strain AD 169 All 19 formed elongated foci composed of round clear cells in primary as well as in passage culture These foci spread very slowly in spite of prolonged incubation and diffuse cytopathic effect was as a rule first seen in the passage cultures

Nuclear as well as cytoplasmic inclusions were seen as part of the cytopathic effect of the isolated strains in hematoxylin–eosin stained preparations

Immunofluorescence studies of these strains revealed a very characteristic picture corresponding to that produced by cultures inoculated with AD 169 Using a known convalescent serum (CF titer 64 NT titer 200) and serum with antibodies against CMV from the patient from whom the virus had been isolated fluorescence was seen in the focal changes in tissue cultures demonstrating the cytopathic effect but not in the non affected cells There was only a very weak fluorescence of these foci when the routinely used negative serum (CF titer <2 NT titer <2) was employed and neither this serum nor the positive sera produced specific fluorescence in the non inoculated cultures

The positive standard serum resulted in a very characteristic fluorescence pattern in the cells infected by all the isolated strains The nuclear inclusions which were of varying size according to the degree of infection in affected cells stood out very sharply while the rest of the cells especially the cytoplasm fluoresced much more weakly

The pattern of fluorescence in the cultures

Table V *Studies in seven patients with cytomegalic cells in the lungs*

Pat no	Histologic diagnosis	Died post transplant day	Serology CF antibody	
			Day	Titer
7	Sporadic cytomegalic cells	335	−2	32
			+17	256
			+257	128
			314	3[illegible]
8	Focal cytomegalic alveolar pneumonia	35	+19	16
			+34	16
11	Several cytomegalic cells diffuse in the lungs	1[illegible]5	−54	16
			−9	8
			+78	[illegible]
			+100	64
			+125	128
19	One cytomegalic cell	34	−30	64
			7	32
			14	32
[illegible]9	Sporadic cytomegalic cells	3[illegible]9	−[illegible]	<2
			+41	2
			+56	32
			+78	256
			+303	128
3[illegible]	Focal cytomegalic alveolar pneumonia	39	−2	[illegible]
			+27	2
			+36	<2
3	Extensive diffuse cytomegalic pneumonia	47	+13	2
			47	64

treated with positive serum from the patient from whom the strain had been isolated was usually of similar appearance but sera from some of the patients produced a strong fluorescence of the nuclear membrane and the cytoplasm while the nuclear inclusions fluoresced only weakly or not at all

Studies of the CF antigen of five of the newly isolated strains were carried out with several screenings and parallel titrations using a series of known positive and negative sera some of which had been obtained from patients with known sero conversion The results of these studies are shown in Table IV

All the strains of Herpes simplex virus isolated in this study could be neutralized by an immune serum produced in rabbits

Identification of three virus strains which apparently belong to the group of adenoviruses is not yet complete These strains grow slowly in HEL and HEM cells but rapidly produce a diffuse cytopathic effect in HeLa cultures Basophilic nuclear inclusions typical of adenovirus are formed and the virus is ether resistant

Histological studies

Lung sections were available from 17 patients Cytomegalic cells were found in seven

Cytomegalic cells are very characteristic They are significantly enlarged 30 to 40 μ in diameter and contain a large round nucleus which is almost completely filled by a homogeneous inclusion body Between this body and the nuclear membrane a narrow clear halo is usually seen Cytoplasmic inclusions of the type seen in virus infected tissue cultures were not seen in the cytomegalic cells observed in this series

The distribution of cytomegalic cells in the lungs varied greatly from patient to patient In some patients several cells were found in a single small area while in others they were seen diffusely throughout the lungs As acute and chronic changes (broncho-pneumonic vascular with bleeding etc) are very common in the lungs from kidney recipients it may be rather difficult to evaluate the possible significance of the CMV infection In addition other studies (7) have shown that there is no direct relationship between the number of cytomegalic cells and the amount of virus

By reviewing the individual lung sections and by comparing sections however information can be obtained that gives an impression of the severity of the infection In patient T 32 cytomegalic cells in rather large numbers were found in association with a single alveolus or several close lying alveoli The cells lay along the alveolar septa like large epithelial cells In another case (T 8) likewise with marked focal changes a lymphocytic infiltration was seen which masked the structure of a dozen alveoli Several typical cytomegalic cells were found among the lymphocytic cells In a third patient (T 33) cytomegalic cells were seen in large numbers along the alveolar septa with one or more cells in almost every alveolus Only single isolated cytomegalic cells were found in the other four patients in T 19 only one was found

The histologic findings together with the time of death after transplantation and the results of the serologic tests from the seven patients are given in Table V As can be seen CMV pneu

monia localized or diffuse was found in patients who died relatively soon after transplantation (35 to 47 days) The other patients in whom only single cytomegalic cells were seen diffusely spread throughout the lungs lived somewhat longer (125 to 335 days) CF antibody was present at death in six of the seven patients and in four an anti body rise was demonstrated in the post transplant period (Table V)

The ten patients in whom cytomegalic cells were not found in the lung sections died at vary ing periods after transplantation two in the first month two in the second two in the third two in the fourth one in the ninth and one in the twentieth month post transplant Serologic studies in six of these patients showed a significant rise of antibody titer in four and no demonstrable rise in two No sera were available from the other four patients who died from 18 to 81 days after transplantation

The results of this study indicate that CMV can produce specific pneumonic changes Changes are particularly marked in the patients who died in the period after transplantation in which the majority of the patients in our series (90% in the prospective study) demonstrate a rise in CF anti body A clinical syndrome was seen during this period in three patients in the prospective study that suggests a generalized infection (see below) Cytomegalic infection may apparently take a chron c course in the lungs as cytomegalic cells were found in the lungs of one patient (T 29) 273 days after a significant rise in CF antibody had been demonstrated (see Table V)

Clinical symptoms in connection with CMV infection

In none of the seven patients in whom cytomegalic cells were found in the lungs on autopsy was death considered to have been caused or sig nificantly contributed to by the CMV infection Six of these patients had symptoms and radiologic signs of pneumonia at death but in every case autopsy findings revealed clearcut evidence of ex tensive bacterial pneumonia Distinct nodular den sities were seen on X ray in two of these patients but autopsy did not provide sufficient informa tion to determine whether these densities were caused by the CMV infection or by the bacterial pneumonia

Patients in the prospective study were ques tioned and examined as soon as a significant rise in CF antibody was ascertained Five of these 17 patients developed clinical symptoms within the month preceding antibody rise which may have been caused by the CMV infection Two of them had only a few days of unexplained low grade fever but the other three presented a clinical syndrome with prolonged fever malaise relative lymphocytosis and slight leucopenia Two had a dry cough at the beginning of the illness and increased pulmonary vascular mark ings were seen on X ray in one The case histo ries of the three patients will be briefly reviewed

T 29 (Fig 2)

An 18 year-old girl with chronic glomerulonephritis who received a kidney from her father in March 1967 The operation was performed without complications Evidence of a mild rejection crisis appeared on the second post operative day and prednisone therapy was started There was a more severe rejection crisis in the fifth post operative week with sodium retention, weight increase decreasing renal function and significant lymphocyturia This crisis, as the first, responded to prednisone therapy An average daily dose of 1 35 mg prednisone per kg body weight was given during the first post transplant month and 0 91 mg/kg and 0 39 mg/kg during the second and third month Forty three days after trans plantation the patient developed fever which continued until the seventy first day Fever was inconstant the temperature was almost normal during the early morning and between 38 and 40 C in the evening. There was headache mild nausea and malaise during the first week of fever A dry cough was present but the chest was clear to percussion and auscultation Chest X ray revealed increased pulmonary vascular markings films taken be fore and after this febrile illness were interpreted as normal. There was a fall in leucocyte count together with a relative lymphocytosis Serum glutamic oxalacetic transaminase measured 46 units 9 days after the onset of fever and was below 3_ units a week later There was no evidence of graft rejection during this period other than fever and graft function remained good throughout with a creatinine clearance of 50 to 80 ml min. Serological tests for ornithosis and toxoplasmosis were negative CMV CF titer two days before fever appeared was less than 2 Two weeks later it was 3_, and three weeks thereafter the titer was 256 Virus neutralizing antibody began to rise 78 days after trans-plantation It was possible to culture CMV from the urine on days 254 and _62 post transplant. A chronic vascular allograft reaction developed nine months after transplantation which proved impossible to control and the patient died in uremia 3_9 days after renal allo-transplantation. Cytomegalic cells were found in the lungs on autopsy and it was possible to culture from lung tissue

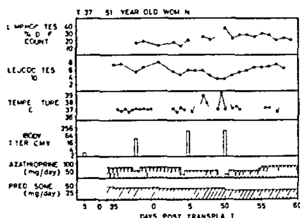


Fig. 3 Post transplant course of patient T 37 with a febrile illness in association with rise of complement fixing antibodies against cytomegalovirus

T 37 (Fig. 3)
A 51 year-old woman with chronic pyelonephritis who received a kidney from her sister in November 1967 Minimal signs of rejection in the form of fever and graft tenderness appeared on the second post-operative day and prednisone therapy was started with 150 mg/day Prednisone was thereafter reduced stepwise the patient receiving in the first three months after transplantation an average daily prednisone dose of 1.20 mg/kg 0 6 mg kg and 0 33 mg kg respectively Graft function was good during the entire post-operative course with ne clearance of 40 to 60 ml/min and there were further rejection crises The patient became febrile on the forty fifth post-operative day and remained so during the following six days with almost normal temperature in the morning and 38 to 39 5 C in the evening At the start of the fever the patient complained of a dry cough but pulmonary stethoscopic findings and chest X ray were normal From post transplant day 47 to 53 there was slight leucopenia 3500 to 5000 leucocytes/μl accompanied by a slight relative increase in the percentage of lymphocytes on differential count The peripheral blood did not contain typical Downey cells or an increased percentage of monocytes but most of the lymphocytes were slightly atypical with increased basophilia and about of them had nuclei containing nucleoli On day 50 the serum glutamic oxalacetic transaminase was 60 units, five days later it was less than 35 units CMV CF antibody five days before transplantation was <2 on post transplant day 31 it was 32 Titer has remained elevated through day 124 (see Table II) *Serologic tests for toxoplasmosis influenza ornithosis* and infectious mononucleosis (Paul Bunnell) were all negative

T 40 (Fig. 4)
A 21 year-old woman with congenital hypoplastic kidneys who received a kidney from her mother in January 1968 There were no operative complications but the post-operative course was characterized by ureteral problems and urinary tract infection. There was a very *mild rejection episode two days after transplantation* with fever but no reduction of graft function. Prednisone was started at that time and the average daily dose was 1 75 mg/kg 0 71 mg/kg and 0 47 mg/kg during the first *three post transplant months There was no further* evidence of graft rejection and renal function has remained excellent throughout the post-operative course with a creatinine clearance of 70 to 80 ml/min On the 83rd post transplant day the patient developed fever chills and malaise after having rhinitis during the preceding week There were no pulmonary symptoms and physical and X ray findings were interpreted as normal Fever which was septic in character—high in the evening low in the morning—lasted for 14 days This temperature elevation was at first thought to be due to a urinary tract infection but streptomycin was without effect and urinary sediment and bacteriology were the same before the fever as after Leucopenia with white cell counts below 5000 cells/μl developed on the 88th post transplant day and continued for about two weeks Lymphocytosis appeared on day 100 and lasted for 24 days Monocytosis was not seen The majority of these lymphocytes which made up from 40 to 80% of the differential count were atypical having the characteristics of the virocytes seen with several viral infections Serum glutamic oxalacetic transaminase was not elevated Paul Bunnell ornithosis CF cold agglutinins toxoplasmosis CF Sabin dye test and tests for influenza CF antibodies were all negative Significant rise in CMV CF antibody was first demonstrated in serum removed on post transplant day 103 when a titer of 128 was found Titer on day 95 had been <2 There were again transient episodes of unexplained fever on post transplant days 133 146 155 165 and 169 In most cases chills preceded the fever which was accompanied by a sensation of nasal stuffiness A fall in leucocyte count to about 4000/μl with relative lymphocytosis was seen from post transplant day 150 to 154

On review of the case records from the 12 patients in the retrospective study in whom significant antibody rises were demonstrated only one patient was found who presented signs and symptoms that may have been due to CMV infection This was a 32 year-old man T 10 in whom sero conversion took place between post transplant day 9 and 45 This patient had fever from day 15 to 43 which at the time was thought to be the result of esophageal moniliasis

DISCUSSION

In the work reported here we have attempted to study the problem of CMV infection in kidney transplant recipients as comprehensively as possible In general we have employed the same methods of study as used by previous investigators (3 13) but we have had more serum samples available from each patient and in addition we have undertaken studies of NT antibody

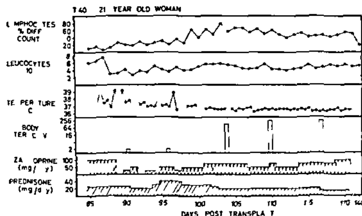


Fig. 4. Post transplant course of patient T 40 with a febrile illness in association with rise of complement fixing antibodies against cytomegalovirus.

Experience with certain aspects of the techniques employed will be discussed briefly. It became quite evident that flask cultures were superior to cultures in roller tubes for the isolation of CMV from the urine and blood of adults. Morphologically the tube cultures were generally good after 60 days of incubation, but only once was it possible to isolate CMV in tube cultures from an adult. In this case it proved necessary, however, to transfer the virus to flask culture in order to save the isolated strain. In flask cultures cytopathic effect can usually be observed in the course of one month after inoculation with virus-containing material, and diffuse cytopathogenic changes often develop rapidly after passage.

The immunofluorescence reaction has been a significant help in the diagnosis of CMV strains. This test was not absolutely necessary, as all of the isolated CMV strains formed characteristic nuclear and cytoplasmic inclusion bodies as part of the cytopathogenic changes. As a serologic technique the immunofluorescence reaction is superior to the study of a strain's CF antigen because it can be carried out less than a week after the first passage of a strain while it takes one to several months to produce a potent CF antigen from a newly isolated strain.

Measuring the titer of NT antibodies by the plaque reduction technique of Plummer and Benyesh-Melnick (11) has, as far as we know, not previously been used in studies of CMV infection. Our experience with this test has been good. We have studied paired sera from ten kidney transplant recipients as well as from three previously healthy adults with CMV infection (15) and were able to demonstrate virus NT antibodies against strain AD 169 in all patients. In all but one we were able in addition to demonstrate a significant rise in NT titer, which in some patients did not begin until 1 to 2 months after the rise in CF titer had taken place.

Preliminary unpublished work on the relationship between CF and NT antibodies in sera from about 50 children and adults has shown that it is generally possible to demonstrate NT antibodies against AD 169 in sera with CF antibodies by this technique. In some CF negative sera NT antibodies were found. These findings are similar to those of Carlstrom (1) and Carlstrom et al. (2) using a tube NT test with AD 169.

We have not as yet had the opportunity to test for the presence of NT antibodies against CMV strains other than AD 169.

CMV infection is characterized by the fact that patients excrete virus in saliva and urine. In this study we have mainly concentrated on the study of viruria, but we did demonstrate in one patient that virus can be isolated from the blood prior to antibody rise. Isolation of CMV from the blood has been reported previously from patients with leukemia (4) and postperfusion CMV infection (9). It may also be a useful test in the diagnosis of CMV infection in patients treated with immunosuppression. Herpes simplex virus was isolated several times, but the level of CF antibodies against this virus did not differ much from the normal. Nor was it specifically influenced by immunosuppressive therapy. Adenoviruses were isolated from the urine of three pa-

tients during a very short period We have not yet undertaken serological investigations but the isolation of only three strains during this study indicates that this virus does not play as impor tant a role as CMV in renal allograft recipients

A high frequency of CMV infection in kidney transplant recipients treated with immunosuppres sive drugs was documented in 1967 by two groups of American investigators (3 13) We have found a frequen y of about 90% The infection may appear relatively soon after the start of immuno-suppressive therapy although the time of infec tion varied from patient to patient In some there was serologic evidence b fore the end of the first post transplant month while in others infection appeared during the third or fourth month or even later In the majority however active infection occurred during the second month after trans plantation It was also in this month that evidence of infection was usually seen in some cases pre senting as CMV pneumonia on autopsy in others as a febrile illness Infection may become chronic either in the form of a protracted lung infection characterized by the presence of cytomegalic cells such is in T 29 in whom cytomegalic cells were und on autopsy 273 days after a significant anti y rise and as a prolonged viruria such as demonstrated in cas T 16 in which virus a isolated from the urine 717 days after anti body ri e

In general elevated CF antibody titers were seen in th se patients for long periods after trans plantation but they have disappear d from the serum of one patient (T 27) in spite of persistent viruria which may perhaps mean that in this pa tient infection was lo alized to the graft Just as Craighead et al (3) we find that CF antibody titer is higher in transplanted patients treated with immunosuppression than in previously healthy in dividuals with CMV infection however we do not find as the above mentioned investigators did that it is common for the level of CF antibody to decrease prior to death In our study we have seen in several patients with CF antibodies in the pre transplant sera that a fall in titer may occur just after institution of the immunosuppressive therapy prior to rise in titer

Among the 43 patients studied there was a remarkable number with CF antibodies in their serum before transplantation—about double as many as would be expected for the age groups involved In 180 healthy adults between th ages of 25 and 45 years we have found as have others (1) an incidence of about 40% It is perhaps not surprising that our patients had this higher inci dence as all of them suffered from a prolonged illness before transplantation that might have reduced their resistance to viral infection

In most cases CMV infection in the Aarhus series of kidney transplant recipients appeared to be the result of the reactivation of latent infec tion In the pre transplant serum from only five patients was it impossible to demonstrate CF or NT antibodies against CMV strain AD 169 This suggests that the three who developed antibodies had a primary infection with CMV One of these patients (T 29) received a kidney from a donor demonstrating a high CF antibody titer and it is possible that she received CMV together with th donor kidney Other exogenous sources are of course possible

A characteristic febrile illness was seen in three patients in close association with a rise in CF antibody None of them had CF antibody before transplantation but two of them had low titers of NT antibody and infection in these two was presumably the result of reactivation of latent infection In one of the patients (T 29) viruria was demonstrated several times and cytomegalic cells were found in the lungs at autopsy Virus has also been isolated from the urine of another (T 37) while this has not yet been possible in the third This illness with fever malaise and lymphocytosis has not previously been reported as a manifestation of CMV infection complicat ing immunosuppressive therapy but such an ill ness is a common expression of the disease among normal healthy adults (2 8 15) Evidence of acute CMV pneumonia was found on autopsy in three patients (T 8 32 and 33) all of whom had clinical and radiologic signs of pneumonia before d ath However autopsy also revealed extensive bacterial pneumonia in all three and therefore it is uncertain what role CMV played in the symp-tomatology Two patients (T 29 and T 37) had a dry cough at the start of their clinical illness and perhaps they had a CMV pneumonitis Slight serum oxalacetic transaminase elevation was seen in cases T 29 and 37 but jaundice was not ob-served Nodular densities similar to those de scribed by Rifkind et al (13) were seen on chest X ray in two of the patients with autopsy evi

dence of CMV pneumonia but here again it is difficult to ascribe this finding to CMV as both had bacterial pneumonia as well

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ENDOCARDIAL PACING IN ACUTE MYOCARDIAL INFARCTION

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Abstract From 1964 to 1968 seventy seven patients in Ullevål Hospital with acute myocardial infarction were treated with endocardial pacing. Sixty of these patients had A-V block 53 complete and 7 A-V block of second degree

Indication for pacing in the patients with A-V block was Adams Stokes attack in 3 patients, bradycardia in 14 and in 23 prophylactic pacing was performed Thirty three patients (55%) survived and 27 (45%) died Mortality was higher in patients with anterior myocardial infarction (69%) than in patients with posterior myocardial infarction (13%) All the patients with both anterior and posterior myocardial infarction died Patients with bundle branch block had a higher mortality (68%) than patients with narrow QRS complexes (21%)

Two patients died during positioning of the electrode and 19 during treatment with pacemaker Among the 19 fatalities during pacing 5 were due to ventricular fibrillation 1 to heart rupture and 13 to shock or cardiac failure

In 17 patients pacing was started on indications other than A-V block and 13 of the latter (76%) died

Better results in the treatment of A-V block in acute myocardial infarction will probably be achieved by the use of "on demand" pacemakers in order to avoid pacemaker induced ventricular fibrillation and by prophylactic pacing before serious complications after Adams Stokes attacks or bradycardia have developed

Complete heart block is reported to occur in about 8% of cases with acute myocardial infarction (5) and the mortality rate is reported to be high figures of 50% and higher are given (3)

Artificial electrical stimulation of the heart is proposed to improve the outlook for these patients Clinical experience however is still limited and there is no general agreement concerning the practical performance

Our experience concerning cardiac pacing in acute myocardial infarction is reported in the following

MATERIAL AND METHODS

Seventy-seven patients with acute myocardial infarction have been treated by endocardial pacing in the period from May 1964 to August 1968 Fifty three were male average age 65 years, 24 women average age 66 years

Sixty of the patients had A-V block in 17 patients pacing was started on other indications

In the first 4 cases a Cournand bipolar electrode in the others unipolar Elema pacing catheters have been used In 67 cases positioning of the electrode was guided by intracardial ECG recording from the catheter electrode without X ray monitoring, as described elsewhere (1)

Until November 1967 only fixed rate pacemakers were used in most cases the Elema 138 external pacemaker Pacing voltage was usually 2-3 times the threshold value In the last year we have also used a ventricular inhibited "on demand" pacemaker

Patients with A-V block

In 60 patients the indication for pacing was A-V block. Age and sex distribution in this group appear from Table I Average age of the men was 63 years and of the women 67 years. Fifty three had complete A-V block, seven second degree A-V block The patients with second degree A-V block all had posterior myocardial infarction Wenckebach's periods were observed in 6 patients. In 5 patients a progress was seen from second degree to third degree A-V block, two of them had also bundle branch block.

Location of myocardial infarction

If information obtained at autopsy is added to the ECG information, it appears that 13 patients had isolated anterior myocardial infarction, 31 isolated posterior myocardial infarction, 2 lateral myocardial infarction 8 both anterior and posterior myocardial infarction 1 anterior and lateral 1 posterior and lateral and 1 anterior lateral and posterior myocardial infarction (Table II) In 3 patients the location of the myocardial infarction is unknown

Bundle branch block

Wide ventricular complexes (QRS duration 0 10 sec or more) was seen in 31 patients after the appearance of A-V block In the period before development of the A-V block bundle branch block was present in 9 patients, not present in 12, and in 10 no ECG recording was available from this period

Two patients with posterior myocardial infarction had intermittent bundle branch block In 2 patients with

Table I. *Patients with A-V block in different age-groups*

Age	Male Total	Male Deaths	Female Total	Female Deaths	Sum Total	Sum Deaths
-49	5	1	0	0	5	1
50-59	12	2	3	1	15	3
60-69	13	8	8	4	21	12
70-79	12	7	5	2	17	9
80-	2	2	0	0	2	2
Sum	44	20	16	7	60	27

infarction of both the posterior and anterior part of septum, bundle branch block developed some hours after the appearance of A-V block. In 29 patients bundle branch block was not observed.

Interval between onset of myocardial infarction and development of A-V block

The interval between the onset of myocardial infarction and development of A-V block was less than 24 hours in 32 patients, 1-2 days in 18, 2-7 days in 9, and unknown in one. Among 13 patients with anterior infarction about one half had developed A-V block within 24 hours; of 25 patients with posterior myocardial infarction two thirds had A-V block within 24 hours (Table III).

Table II. *Mortality in relation to bundle branch block, location of myocardial infarction and indication for pacing*

In brackets deaths in the different groups (BBB = bundle branch block)

	BBB observed	BBB not observed	Sum
All cases	31 (21)	29 (6)	60 (27)
Location of myocardial infarction			
Anterior	12 (8)	1 (1)	13 (9)
Posterior	6 (1)	25 (3)	31 (4)
Lateral	1 (1)	1 (1)	2 (2)
Anterior and posterior	8 (8)	0	8 (8)
Anterior and lateral	1 (1)	0	1 (1)
Posterior and lateral	0	1 (1)	1 (1)
Anterior, posterior and lateral	1 (1)	0	1 (1)
Unknown	2 (1)	1 (0)	3 (1)
Indication for pacing			
Adams-Stokes attack	17 (17)	6 (2)	23 (19)
Bradycardia	8 (5)	6 (2)	14 (7)
Prophylactic	6 (4)	17 (2)	23 (6)

RESULTS

Indication for pacing

The indication for pacing was Adams-Stokes attacks in 23 patients. In nine of these patients the attacks were not recorded on ECG; in the remaining 14 patients ventricular standstill or bradycardia was registered. Fourteen patients had ventricular bradycardia but no Adams-Stokes attacks. Twenty-three patients with A-V block grade 2 or 3 had neither Adams-Stokes attacks nor bradycardia, and the pacemaker was inserted prophylactically to prevent such episodes. The incidence of Adams-Stokes attacks was greater in patients with bundle branch block (55 %) than in those without (21 %). Five patients with posterior

Table III. *Mortality related to the interval between onset of myocardial infarction and development of A-V block*

In brackets deaths

	A-V block developed within 24 hours	A-V block developed after more than 24 hours	Interval between onset of infarct and A-V block unknown
Total A-V block	31 (13)	21 (13)	1 (0)
Second degree block only	1 (0)	6 (1)	
All cases	32 (13)	27 (14)	1 (0)
Location of infarct (second degree block excluded)			
Anterior	6 (3)	7 (5)	
Posterior	16 (2)	9 (2)	
Lateral	1 (1)	1 (1)	
Anterior and posterior	6 (6)	2 (2)	
Anterior and lateral	0	1 (1)	
Anterior, posterior and lateral	0	1 (1)	
Posterior and lateral	0	1 (1)	
Unknown	2 (1)	0	1 (0)

myocardial infarction and narrow QRS complexes gave a history of one or more syncopes which occurred before the patients arrived in the hospital One of these patients was unconscious on admission to hospital and died shortly afterwards

Ventricular fibrillation during pacing for A–V block

Ventricular fibrillation occurred in 11 patients with A–V block In ten of these patients ventricular fibrillation was observed during effective pacing Nine had fixed rate pacemakers and one on demand pacemaker Five died during or shortly after the episodes Previous thereto most of the patients were seriously ill one had attacks of ventricular fibrillation before pacing was started Autopsy revealed large myocardial infarctions including both the anterior and posterior part of the septum in two and the anteroseptal area in the other three

In four patients ventricular fibrillation was directly provoked by the pacemaker equipment one patient had ventricular fibrillation when the electrode was manipulated during positioning two had an attack of ventricular fibrillation when accidentally paced by two pacemakers during measurement of threshold value shortly after the electrode had been positioned and one patient had repeated short bouts of ventricular fibrillation during the first hours of pacing when the pacemaker impulse fell at the end of the T waves These four patients survived

Survivals

Thirty three patients (55 %) left the hospital alive In at least five of these patients the symptoms directly related to the A–V block were very severe and were promptly relieved by the pacing We believe the cardiac pacing saved the lives of these patients

Only one patient was discharged with a permanent pacemaker This patient had an anterior myocardial infarction and bundle branch block

Deaths

Of the 60 patients with A–V block 27 (45 %) died Two patients died during positioning of the electrode before cardiac pacing was established They were both extremely ill unconscious and in shock

Table IV *Indications for pacing other than A–V block*

Indication	No pats	Deaths
Cardiac standstill	9	8
S–A block	1	0
Nodal bradycardia	1	1
Sinus bradycardia	2	0
Atrial tachycardia with block	1	1
Ventricular tachycardia	1	1
Ventricular fibrillation	2	2

Nineteen patients died during pacemaker therapy Twelve of them were in an extremely bad condition before the therapy was started with hypotension and cardiogenic shock The ultimate cause of death was ventricular fibrillation in five cardiogenic shock in ten congestive failure in three and myocardial rupture in one

Six patients died after pacing had been stopped four of them in congestive failure Before equipment for constant cardiac monitoring was available in the hospital one patient in whom pacing had been stopped after a few days was found dead After this episode we have as a rule kept the pacing catheter in place for three weeks and we have never stopped pacing during this period unless the patient was under continuous monitoring thus making it possible to resume pacing immediately if necessary One old demented patient pulled out the electrode and pacing was not resumed He died suddenly after 18 days

Autopsy

Autopsy was performed in 25 of the 27 fatal cases In 13 the myocardial infarction was extensive affecting approximately one third or more of the left ventricle In the remaining 12 the extension of the myocardial infarction was moderate

Isolated posterior myocardial infarction was only seen in four of these 25 cases All of them died in cardiogenic shock

In two cases the electrode was found in the coronary sinus Perforation of the heart by the electrode was not found in any case

Prognostic factors

Of 31 patients with isolated posterior myocardial infarction four died (13 %) of 13 with isolated

anterior infarction nine died (69%) Ten patients with anterior infarction in combination with other locations all died

Of seven patients with A–V block of second degree only one died while of 53 patients with complete A–V block 26 died (49%)

Of 31 patients who had bundle branch block 21 (68%) died (Table II) Among 29 patients in whom bundle branch block was not observed six (21%) died Among patients with ECG evidence of isolated posterior myocardial infarction and bundle branch block mortality was 17% against 12% in patients with narrow QRS complexes

Mortality related to the indication for pacing appears from Table II The mortality was far higher in patients who had Adams Stokes attack (61%) than in patients on prophylactic pacing (30%)

In patients with total A–V block the mortality was 41% when the A–V block appeared within 24 hours after onset of the myocardial infarction and 62% in patients who developed A–V block later In half the number of patients with anterior myocardial infarction the A–V block developed late and the prognosis in these patients was extremely poor (Table III)

ing on indications other than A–V block

n 17 patients with acute myocardial infarction endocardial pacing was started on indications other than A–V block These patients who form a heterogenous group will be mentioned only briefly In nine of the patients the indication for pacing was cardiac standstill i e circulatory arrest with no atrial or ventricular activity in the electrocardiogram This occurred after defibrillation for ventricular fibrillation in 4 patients The other indications appear from Table IV Thirteen of these patients (76%) died

Ventricular fibrillation during pacing was seen in three of the patients In one patient with sinus bradycardia an attack of ventricular fibrillation was triggered when the pacemaker was started Pacing was stopped the patient defibrillated and survived Two other patients died after attacks of ventricular fibrillation In one of them the indication for pacing was recurrent attacks of ventricular fibrillation the other was paced after nodal bradycardia All these patients were on fixed rate pacing

DISCUSSION

The high mortality in patients with acute myocardial infarction and A–V block will in many cases be directly related to the conduction disturbance On the other hand many of these patients have a severe coronary artery disease with extensive myocardial infarctions A large proportion of the fatalities therefore are inevitable and due to extensive destruction of the myocardium But as the present report has shown, cardiac pacing in acute myocardial infarction obviously may save some patients

In this material as in others dealing with patients on pacemaker therapy mortality was greatest in patients with anterior myocardial infarction probably due to the great extension of the infarction (4) In patients with isolated posterior myocardial infarction the prognosis is far better Lassers and Julian found a mortality of 27% in patients with posterior myocardial infarction and 83% in patients with anterior infarction (6) Patients with isolated posterior myocardial infarction and bundle branch block have a higher mortality than patients with narrow QRS complexes This is in accordance with observations made by others (6) Patients with anterior myocardial infarction usually have bundle branch block when the A–V block appears

Patients with acute myocardial infarction are more liable to develop ventricular fibrillation than other patients In paced patients this coomplication may be provoked by mechanical or electrical stimulation from the electrode The risk of causing ventricular fibrillation therefore has to be weighed against the positive results obtained by the treatment of the heart block Normally the threshold for provoking ventricular fibrillation is 10–30 times the threshold for ventricular stimulation (11) Myocardial ischemia will lower the fibrillation threshold considerably as demonstrated in experiments on dogs by Wiggers et al (13) A patient with acute myocardial infarction is therefore probably more liable to develop a pacemaker induced ventricular fibrillation than other patients Ventricular fibrillation is most liable to occur in patients with competition between sinus rhythm and pacemaker induced rhythm because many pacemaker impulses will then fall in the vulnerable phase at the end of the T wave Therefore patients with acute myocardial infarction should preferably be paced by

a ventricular inhibited on demand pacemaker Such pacemakers are now available commercially

In some cases it has been observed that the amplitude of the intracardial potential picked up by the pacing electrode is too low to inhibit the demand pacemaker which will then work at a fixed rate (12) If pacing has to be done at a fixed rate the lowest possible pacing stimulus should be used The pacing threshold is altered by eating sleeping and by many drugs variations up to 30 % are observed (9 11) If fixed rate pacing has to be used in acute myocardial infarction the pacing stimulus should therefore probably exceed the threshold value by 30–50 % During the first days the pacing threshold will normally increase and the stimulus has to be adjusted accordingly

We have used the soft and flexible Elema unipolar pacing catheter These catheters are sometimes difficult to position To position the catheter the patient invariably has to be turned on the left side A stiffer catheter or a catheter with a guiding stylette is easier to insert especially if TV monitoring is available and turning of the patient is not necessary With a stiff catheter however there is a risk of perforating the right ventricle Therefore as a routine method to be used by physicians without previous experience in cardiac catheterization a soft catheter is preferable Positioning of catheters during TV monitoring is the method of choice but insertion guided by intracardiac ECG can safely be used when TV monitoring is not available

The alternative to electrical stimulation is pharmacological therapy In acute A–V block the infusion of isoprenaline is usually effective also in patients with myocardial infarction We have used isoprenaline infusion before placement of the pacemaker in our patients but we have not relied on this as the only therapy Disadvantages of isoprenaline in these patients are the tendency to provoke ectopic tachyarrhythmias and the forceful drive on the heart with oxygen wasting effect in the myocardium

The real reduction in mortality which is achieved by endocardial pacing in A–V block is difficult to assess In 67 patients with advanced heart block Cohen reported a mortality of 75 % in anterior myocardial infarction and 37 % in diaphragmatic myocardial infarction (2) Average mortality in 17 reported series of patients with advanced heart block was 58 % (10) Mortality rates in hitherto published reports on paced patients are slightly lower but comparison is difficult due to selection of patients (6 8 10 12)

In our opinion cardiac pacing ought to be instituted in patients with anterior myocardial infarction and advanced second degree or complete A–V block The prognosis in this patient group is poor due to the usually very extensive myocardial infarction However an Adams Stokes attack or extreme bradycardia due to A–V block most likely will make the condition even worse Some authors also advocate prophylactic endocardial pacing in patients with anterior myocardial infarction and left bundle branch block as these patients are liable to develop a sudden total A–V block (7)

In patients with posterior myocardial infarction and narrow QRS complexes the prognosis is good We have however observed Adams Stokes attacks in such patients and we therefore believe that endocardial pacing ought to be instituted when complete A–V block occurs even if the patient has a satisfactory heart rate The natural history of the second degree A–V block in patients with posterior myocardial infarction and narrow QRS complexes is at the present time not known Patients with posterior myocardial infarction and second degree A–V block with Wenckebach s periods might be kept under observation without pacemaker in a coronary care unit with facilities for emergency therapy

We believe that the results achieved by endocardial pacing in acute myocardial infarction will be improved by the use of on demand pacemakers in order to avoid pacemaker induced ventricular fibrillation by reducing the pacing stimulus to the lowest possible level if fixed rate pacing has to be used and in patients with advanced A–V block by prophylactic pacing to avoid complications such as Adams Stokes attacks or severe bradycardia

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A CASE OF KLINEFELTERS SYNDROME WITH 48,XXXY AND DIABETES MELLITUS

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Abstract The twentieth case of Klinefelters syndrome with 48 XXXY in a 39 year-old Caucasian is described. The outstanding clinical feature was a diabetes of the maturity onset type of nine years duration but with a somewhat slow plasma insulin response and an early peak of plasma growth hormone

The patient was slightly dysphoric and immature with no sexual libido or potency and with a total I Q of 74 During a six months course of testosterone his general condition improved and alterations in his mental and sexual condition were also noted

Cell from buccal smears and fibroblasts from the testis and the skin cultures were sex chromatin positive with a considerable number of double positive nuclei In 209 analysed metaphases 78 had the modal chromosome number of 48 with a karyotype of 48,XXXY whereas 72% were non modal cells, preferentially with a hypomodal chromosome number Analysis of metaphases with one missing chromosome indicated random loss of chromosomes except in cells from testis biopsy where the lacking chromosome belonged to the group 21-22-Y which makes a testis mosaicism possible

PATIENTS WITH KLINEFELTERS SYNDROME 48,XXXY

Nineteen cases of Klinefelters syndrome with 48,XXXY have previously been described four from Canada by Barr et al (1) and Carr et al (5) two from USA by Ferguson Smith et al (8) and later by Money and Hirsch (25) and five from Great Britain by MacLean et al (19) and Hunter (15) Three such patients have been described in Japan one by Makino et al (20) and a pair of monozygotic twins by Takayosu et al (31) One patient with 48 XXXY has been described in France by Gilly et al (12) two cases from Sweden by Hambert (14) and two from Denmark by Nielsen and Fischer (28) and Frøland (11) I Q was below 70 in all patients but the one found by Nielsen and Fischer in 1965 had an I Q of 72 All patients were typical cases of Klinefelters syndrome One was described as schizophrenic and one suffered from a psychogenic psychosis

Mosaics with 47,XXY/48,XXXY have been described in two mentally retarded patients one from Canada by Barr et al (2) and one from USA by Finley et al (9)

DIABETES MELLITUS IN PATIENTS WITH KLINEFELTERS SYNDROME

Single cases with Klinefelters syndrome and diabetes mellitus have been published by Langeron (18) Castleman and Kibbee (6) Yodaiken (36) Benda et al (4) Fraser (10) Rhode (30) Zaninowich (37) and Lamotte (17)

Becker et al (3) found mild diabetes mellitus in five out of 50 patients with Klinefelters syndrome giving a frequency of 10% Jackson et al (16) studied the glucose metabolism in seven patients with chromatin positive Klinefelters syndrome and one patient with chromatin negative Klinefelters syndrome Only one of the eight patients had a family disposition to diabetes mellitus his paternal grandfather had diabetes One of the eight patients had a diabetic glucose tolerance curve and one an increased early insulin response Menzinger et al (21) found normal glucose tolerance curves in eight patients with Klinefelters syndrome

Mirouze et al (24) studied the glucose metabolism in four patients with Klinefelters syndrome and found prediabetic insulin glucose

tolerance values in all four patients Nielsen (27) found that four of ten patients with Klinefelter s syndrome for whom a glucose tolerance test was available had blood sugar of 120 mg/100 ml or higher at two-and a half hours using the Hagedorn Norman Jensen method of glucose determination giving a frequency of 40% Two fathers and three mothers among the parents of 25 patients with Klinefelter s syndrome suffered from diabetes mellitus (10%) Diabetes mellitus was found in the relatives of eight of the 25 patients with Klinefelter s syndrome (32%)

Wais and Salvati (34) found three patients with diabetes mellitus out of 32 patients with Klinefelter s syndrome giving a frequency of nine per cent Four fathers and three mothers of the 32 patients had diabetes mellitus (11 /) Diabetes mellitus was found in the near relatives in eight of the 32 patients (25%)

Zuppinger et al (38) found a diabetic glucose tolerance curve in six of 24 patients with Klinefelter s syndrome giving a frequency of 25% Two of the 24 patients had clinical diab tes mellitus Three fathers and one mother of the 24 patients suffered from diabetes mellitus (8%) and diabetes mellitus was found among near elatives of 11 of the 24 patients (46%) Nielsen al (29) found diabetic glucose tolerance in 12 f 31 patients with chromatin positive Klinefelter s syndrome (39%) All three patients with the chromosome constitution 48 XXXY including the patient presented in the present paper had a diabetic glucose tolerance

HUMAN GROWTH HORMONE IN PATIENTS WITH KLINEFELTER S SYNDROME

Glick et al (13) found low fasting serum growth hormone levels in two patients with Klinefelter s syndrome Nielsen et al (29) found a normal mean fasting plasma growth hormone level in 26 patients with chromatin positive Klinefelter s syndrome but 35% had an early plasma growth hormone peak compared to only 8% in a control group

CASE REPORT

Medical Evaluation

A 39 year-old male who during the preceding ten years had suffered from several attacks of thrombophlebitis was admitted to the hospital (1965/66) because of severe thrombophlebitis of the right leg with several chronic inflamed ulcers on the crus and foot. There was no family history of diabetes but in 1957 at the age of 30 an oral glucose tolerance test was found diabetic The blood sugars at that time were fasting 110 mg 15 212 mg 30 248 mg 45 257 mg 60' 244 mg 90 216 mg 120 175 mg and 150 130 mg all per 100 ml No dietary treatment or insulin was given

On admission the physical examination revealed an eunuchoidal 173 cm tall prematurely aged man with fine wrinkles around the eyes and mouth His weight was 105 kg The facial hair was absent and the pubic and axillary hair was scant The penis was small and the prostata not palpable The right testis was absent. In the left scrotum a small soft structure was felt which in connection with biopsy through an inguinal incision was seen mainly to consist of a normal epididymis The testis proper measured 8 × 10 × 20 mm Histological examination revealed only fibrous tissue with a small island of non characteristic epithelial tissue which might be Leydig cells Hyalinized seminiferous tubules were not present in the biopsy specimen.

On the right foot and the right lower crus were several ulcers from pin size to 40 × 20 mm There was pronounced edema of the region and the skin was dry reddish discoloured and finely desquamating The temperature was occasionally slightly elevated (38 –38 5) during the first week after admission

The remaining physical examination was normal In particular fundoscopy revealed no late diabetic manifestations

Examination of the blood showed a sideropenic anemia with Hb 9 7 g/100 ml Se Fe 26 μg/100 ml and Se transferrin 361 μg/100 ml There were normal electrolytes and no acidosis Electrophoresis of serum showed a slight reduction of the albumin fraction The urinary sediment was normal and Se creatinine 0 9 mg/100 ml X rays of the lungs and heart and an electrocardiogram were normal X rays of the bowel and stomach revealed a large esophageal hiatus hernia which was held responsible for a detected slight bleeding An X ray of the skull was normal The electroencephalogram was slightly abnormal with some 5–7 c/s predominantly over the left hemisphere

Basal metabolic rate protein bound iodine triiodothyronine absorption test and Se-cholesterol were normal Se cortisol showed a normal diurnal variation and ACTH and metopirone tests were normal Pituitary gonadotropin excretion was increased (70–265–220 mouse units/24 h) and Se testosterone decreased (0 2 μg/100 ml) The oral glucose tolerance curve (Fig 1) was diabetic and the fasting morning blood sugar around 250 mg/100 ml There was severe glucosuria but no ketone bodies in the urine The response of plasma insulin during the glucose load was slight corresponding to a moderately severe glucose intolerance and the serum growth hormone showed an early peak at two hours (The assays of plasma insulin and growth hormone were done by Drs K Johansen and H Ørskov Second University Department of Internal Medicin Aarhus Kommunehospital)

Blood coagulation was slightly abnormal The thrombo

plastin activation test and coagulation time were shortened, while the plasminogen concentration was excessively high (355.2 μg Cu Tyrosin/ml) The remaining coagulation tests, the number of thrombocytes the coagel retraction, the recalcification and partial thromboplastin tests the prothrombin (Quick) the prothrombin proconvertin and factor V tests, the plasma thrombin test fibrinogen concentration, euglobulin test and thromboplastin generation test were all within normal limits

Psychiatric Evaluation

The father a smallholder was 39 years old when the patient was born. He suffered from asthma and died after a brain hemorrhage at the age of 75 The mother was 30 years old when the patient was born she is alive and healthy The patient is number three of four siblings he has two brothers and one sister One brother suffers from asthma and the other brother has a tumor on the scrotum. The sister has two healthy children, but four of her children were either stillborn or died shortly after birth two of them were twins

The patient grew up in a good and harmonious home He was very much tied to his mother and he was the most spoilt of the four siblings He never participated in boys play or sports of any type At school he was not doing as well as his siblings he had quite a lot of trouble with arithmetic and writing but he got along quite well with the teachers as well as his schoolmates He left school after the compulsory seven years, and he has stayed at home with his parents since then He has received disablement pension since the age of 35 He and his mother have some home work together packing bandages for the local hospital

The patient never had any erection or pollutions and he never masturbated He has not developed any sexual libido and he never felt like having and never had a girl friend or sexual relations

In the medical ward he behaved very childishly but was happy satisfied friendly and joking He got along quite well with the other patients and he was not teased

At the psychiatric examination he appeared immature quiet, passive and slightly apathetic but he willingly answered the questions He was somewhat dysphoric but not depressed There were no psychotic and no neurotic symptoms.

Psychological testing with WAIS showed a verbal I Q of 74 a performance I Q of 76 and a total I Q of 74 with a comparatively great variation in the results from subtest to subtest There was a pronounced tendency to selfcentered concrete thinking, and his imagination was colored by childish needs.

The personality tests also revealed primitive and oral content in fantasies, and there were further signs of identification difficulties. The childish primitive way of adaptation was supported by defence mechanisms, such as denial and repression, and his conflicts were brought out by questions related to identification.

Course and Treatment

The patient s diabetes was regulated with diet and 36 international units of long acting insulin upon which the blood sugar stabilized within three weeks after admission at 80-270 mg/100 ml during the day and the urine was without or showed only a trace of sugar The patient furthermore received oral administration of iron and diuretics and his ulcers were treated conservatively

After two weeks of observation the patient experienced a new attack of thrombophlebitis of the right leg and developed an enormous edema of the right crus and foot which persisted together with the ulcers. Five weeks after admission the patient suddenly developed fever (39 9 C) an infection with staphylococcus aureus was established and quickly controlled by combined treatment with penicillin methicillin and fusidine

Two months after admission the hemoglobin concentration started to increase but did not reach normal range until after nine months of oral iron medication.

Four weeks after admission treatment with 50 mg every third week of a testosteroneisobutyrate preparation with protracted action was started and continued for six months. Clinically the patient responded to this treatment by developing greater initiative and his general strength and condition improved Within one month an increase in the length of the penis was noticeable and the patient began to experience erections several times a day especially stimulated by the sight of women on TV and he also started to masturbate and experienced a few ejaculations.

Within a week after the start of testosterone treatment the edema of the right leg decreased and the ulcers improved Unfortunately the staphylococcal infection came at this moment and the above mentioned antibiotic treatment was given for seven days After one month only one ulcer over the right medial malleolus remained which however persisted during the observation period of eight months. The attacks of thrombophlebitis terminated

The blood coagulation tests were repeated and were normal one and six months after the initiation of the testosterone treatment, but remained normal two months after the termination of the treatment.

A psychiatric evaluation after five months of testosterone treatment revealed that the patient was more open and talkative and that dysphoric tendencies were no longer apparent The patient had felt the development of sexual lidibo and masturbation disturbing Because the creation of sexual libido with no potency at the age of 39 in such an immature man may be quite dangerous, leading to pedophilia, the testosterone treatment was definitely withheld

Chromosome Analysis

(The cytogenetic part was done by
G Bruun Petersen)

Material and methods Buccal smears for sex chromatin investigation were stained by the Feulgen method using hydrolysis in *5 N* HCl at room temperature for 1 h followed by staining for 2 h.

Leucocytes from peripheral blood were cultured using the method of Moorhead et al (26) Two samples of peripheral blood and two skin biopsy specimens were taken with an interval of six months Skin fibroblasts were grown and later prepared for chromosome analysis

Table I *Number of cells counted and analysed in various tissues*

Tissue	Experiment no	Chromosome count 44	45	46	47	48	49	50	51	Total no of cells analysed
Blood culture 1	PB 265	2		7	14	36		1		60
Blood culture 2	PB 271			1	2	27	1			31
Skin culture 1	H 183			1		28	1			30
Skin ulture 2	H 189					30				30
Left testis	T 13	3	1	4	6	42	1		1	58
Total		5	1	13	22	163	3	1	1	209

by the technique described by Therkelsen (32) as were also fibroblasts from the left testis

Results Buccal smears were sex chromatin positive as 40 of the nu lei contained one and 13 of the nuclei contained two sex chromatin bodies (200 nuclei were examined) Cultures from skin as well as testis biopsies were also sex chromatin positive with a considerable number of double positive nuclei

A growth curve experiment performed with the skin fibroblast cultures has been published earlier (33) It was shown that the frequency of double positive cells rose to close on 100 in the postlogarithmic growth phase Of the 2006 cells examined two cells had three and four cells had four sex chromatin bodies

Chromosomes were counted and analysed in 209 meta s s The results are given in Table I The modal omosome number was 48 in all the cultures In all odal cells (78) the karyotype was consistent with the 48 XXXY complement An example of the karyotype is shown in Fig. 2

Of the aneuploid metaphase five showed a hypermodal chromosome number Two of thes had the karyotype 49,XXXY C+ but one had in addition D trisomy and F monosomy One metaphase had 49,XXXY C+ plus a

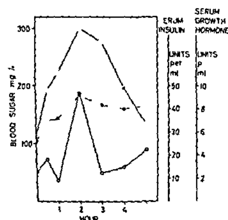


Fig 1 Serum insulin and growth hormone response during an oral glucose toleran e test. x——x Blood sugar ● ● serum insulin O——O serum growth hormone

fragment one had 48 XXXY plus a fragment and the metaphase with 51 chromosomes had several abnormalities

The existence of hypomodal stem lines in blood cultures made possible by the great number of hypomodal cells in this tissue is unlikely when the results in Table II whi h give the analysis of 22 metaphases with th chromosome number $2n = 47$ are considered In cultures from the testis biopsy however all the missing chromosomes belonged to the G group (21-22-Y) Furthermore the analysis of all the hypomodal metaphases in the testis culture showed that among a total of 29 lacking chromosomes 13 were from the G group Estimating the 99 confidence limits for the frequency P of lacking group G chromosomes from our results of 13 lacking G group chromosomes out of a total of 29 we get $0.22 < P < 0.69$ As the P value expected i 5 48 = 0 10 our results seem to indicate a significant departure from random loss of chromosomes in testis culture

DISCUSSION

Generally the clinical manifestations were not different from those presented by patients with Klinefelter s syndrome 47 XXY and a high excretion of pituitary gonadotropin and low Se testosterone were found All patients with Klinefelter s syndrome and 48 XXXY have been mentally retarded with I Q below 75 whereas patients with Klinefelter s syndrome and 47,XXY have a mean I Q around 90 Our patient had a full scale I Q of 74 and he was immature and passive with no sexual libido or potency

The finding of 39 % with diabetic glucose tolerance among patients with Klinefelter s syndrome by Nielsen et al (29) indicates that the chromosome constitution 47 XXY and 48,XXXY predispose to chemical diabetes mellitus and there is most probably a relation between the diabetes mellitus in the present patient and his chromosome constitution of 48 XXXY

The diabetes mellitus of the present patient was

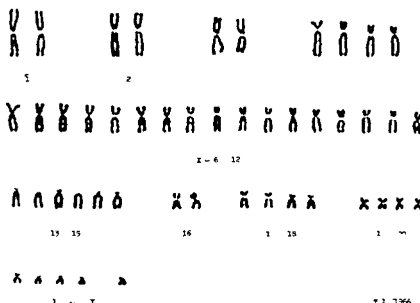


Fig The karyotype from a cell with the 48,XXXY complement.

detected at the age of 30 by an abnormal glucose tolerance curve and the disease ran its course during the following nine years without giving rise to symptoms of glucosuria even when the patient on several occasions was hospitalized with thrombophlebitis In connection with the present admission high blood sugar and severe glucosuria were present without however giving rise to ketonuria or acidosis in spite of a slight fever The patient had not recognized any diabetic symptoms Clinically the diabetes thus appeared to be of the maturity onset type but the patient s response of plasma insulin upon a glucose load was not so delayed and pronounced as is usually seen in maturity onset diabetes The observed early peak in plasma growth hormone is more characteristic of diabetics of the juvenile type (35) but a similar early peak has been observed in 35% of 26 patients with Klinefelter s syndrome (29)

The existence of a slight coagulation abnormality possibly a decreased fibrinolytic activity in a case of XXXY syndrome with nine years of recurring attacks of thrombophlebitis could suggest a causal relationship to the chromosomal abnormality The other two known XXXY patients in this country were therefore investigated but showed completely normal coagulation tests and the coincidence therefore appeared fortuitous

The evaluation of the effect of testosterone treatment on the ulcers of the leg is difficult The ulcers appeared to improve in conjunction with the start of the treatment but they may also have improved because the diabetes had been brought under control even if the patient at the start of the testosterone treatment had received

Table II *Results of analysis of 22 metaphases with one lacking chromosome (2n = 47)*

	Number of cells lacking a chromosome in group						
	X–6–12		21–22 Y		Other		
	Observed	Expected	Observed	Expected	Observed	Expected	Total
Blood cultures	6	5 7	2	1 7	8	8 7	16
Left testis	0	2 1	6	0 6	0	3 3	6

Expected values were calculated on the assumption that all chromosomes have an equal chance of getting lost

insulin for one month without showing any apparent improvement in the ulcers and even experienced a new attack of thrombophlebitis Also the patient received antibiotic treatment for a short period soon after the institution of the testosterone treatment which may have promoted the tendency of healing The coagulation abnormality disappeared upon treatment with testosterone but did not reappear after withdrawal of the treatment It is thus not justified to assume a connection between the testosterone treatment and the disappearance of the minimal coagulation abnormality and the improvement in the leg ulcers

The cytogenetic investigation showed that the patient has a modal chromosome number of 48 in all three tissues examined and in all 163 metaphases with the modal chromosome number the karyotype was 48 XXXY

The frequency of non modal cells was 22 % but the analysis of non modal cells in blood cultures showed random loss of chromosomes A stem line with fewer than three X chromosomes is excluded in skin cultures by the fact that the frequency of cells with two sex chromatin bodies rose to very close on 100 % in the post logarithmic growth phase A stem line with more than three X chromosomes is unlikely on account of the few cells with more than two sex chromatin bodies

In testis however all the $2n = 47$ metaphases had a lacking chromosome in group G Furthermore among a total of 29 lost chromosomes in this tissue there was a significant departure from random loss in the G group ($P < 0.01$) and the possibility of mosaicism in testis must be taken into account. Miller et al (23) have shown that the Y chromosome has a peripheral localisation in the metaphase plate just like the 21–22 autosomes (22) Court Brown et al (7) showed that in males past 65 years the incidence of XO cells increases with age This may give a greater chance of loss of the Y chromosome or of a chromosome in the 21–22 members But as in blood cultures (Table II) there is a good agreement between observed and expected numbers we favor testis mosaicism as the most likely explanation

The question of whether the lacking chromosome is a Y chromosome or a 21–22 autosome cannot be resolved as the patient s Y chromosome shows no identifiable characteristics (Fig 2)

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Acta med scand Vol 186 pp 35–38 1969

STUDIES IN URINARY TRACT INFECTIONS

I The Diagnosis of Bacteriuria in Women

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Abstract Bacterial counts of successively collected mid stream urines and of urine obtained by suprapubic needle aspiration are compared Bacterial counts in 29% of the patients with bacteriuria showed fewer than 10^5 organisms per ml It is concluded that with regard to the number of organisms, no definite boundary exists between contamination and counts indicating bacteriuria with sufficient certainty

More than 10^5 bacteria per ml in two consecutive mid stream specimens or more than 10^6 in a single specimen indicates bacteriuria If fewer than 10^4 organisms per ml are found in a single concentrated clean voided urine specimen bacteriuria may be excluded Bacterial counts in the range of 10^4 to 10^6 in a single mid-stream specimen from a woman do not permit a diagnosis of bacteriuria with sufficient certainty

Demonstration of bacteriuria i e "a state in which active growth is occurring in the urinary passage" (2) is essential for the diagnosis of urinary tract infection The introduction of quantitative urine culture in clinical practice (5 8 12) has largely made it possible to distinguish between bacteriuria and and contamination in clean voided urine specimens The use of percutaneous needle aspiration of the bladder (9) makes it possible to collect specimens aseptically This permits the diagnosis of bacteriuria regardless of the number of organisms found

The term "significant bacteriuria" is generally used when bacterial counts in clean voided specimens indicate bacteriuria with a degree of certainty sufficient for clinical practice Too little attention has often been paid to the dependence of the criteria for significant bacteriuria upon the persons examined the methods of urine collection and the number of specimens cultured

In the present study bacterial counts on successively collected mid stream specimens and on urine aspirated by suprapubic puncture have been compared

MATERIAL AND METHODS

Over a period of eighteen months bacterial counts were made on urine specimens obtained from 458 non pregnant women between the ages of 16 and 65 These women were referred to the out patient clinic of the Roskilde County Hospital because of symptoms of urinary tract infection One urine specimen was obtained from each patient on each of two consecutive days In 217 cases a third specimen was examined

Before the collection of the urine the patients were carefully instructed by a nurse who washed the vulva with sterile water using sterile cotton wool swabs The last of these was left in the vagina The labia were held apart by the patient, and approximately half way through the micturition a sterile plastic cup was introduced into the stream of urine (14) The urine was decanted into sterile tubes and immediately stored at 4 C

During the last twelve months of the study suprapubic bladder puncture was attempted at the second visit of all new patients who had not urinated during the last two hours before arrival The puncture was performed with the patient in the supine position The skin was disinfected with iodine and the puncture was made in the midline about 3 cm above the symphysis pubis using an 8 cm long needle no 8 ("intramuscular needle") attached to a 10 ml syringe The needle was passed through the abdominal wall and directed toward the pelvic cavity No local anesthetic was used 6 to 8 ml of urine were aspirated from each of 95 women

Quantitative bacterial culture of the urine was performed using the calibrated loop technique on nutrient agar and on blood agar plates and by streaking of the inoculum with a sterile glass rod (4) The plates were examined after 24 and 48 hours at 35 C

RESULTS

A comparison of the bacterial counts of mid stream urine specimens obtained from the same

Table I *Comparison of bacterial counts of two consecutive mid stream urine specimens from 458 women*

First specimen Organisms/ml	No of pats	Second specimen Organisms/ml $<10^3$ ( )	10^3–10^4 ( )	10^4–10^5 ( )	10^5–10^6 ( )	$>10^6$ ( )
$<10^3$	93	47	43	4	6	0
10^3–14^4	121	41	47	4	4	3
10^4–10^5	38	16	18	32	13	21
10^5–10^6	55	11	13	14	26	36
$>10^6$	151	0	3	2	16	79

pati nt at different times showed that the count of a second and a third specimen agreed with the first count if fewer than 10^4 or more than 10^6 organisms per ml were found in the first specimen However in four of 42 women with fewer than 10^4 in two consecutive mid-stream specimens bacteriuria was demonstrated by culture of urine aspirated by suprapubic puncture

The probability of error in accepting results in the range of 10^4 to 10^6 organisms per ml in a single specimen was found to be substantial (Table I) but the demonstration of 10 to 10^r two consecutive clean voided specimens brings the agreement with a third specimen up to approximately 90 % (Table II) In all of 29 patients with more than 10 in two consecutive mid stream specimens bacteriuria was demonstrated in urine obtained by suprabupic puncture

In 46 patients the aspirated urine was sterile In two cases mid stream urine collected in connection with the puncture contained more than 10 organisms per ml (Table III) but in neither case did the bacterial count agree with the count of another mid stream specim n from the same patient

Table III *Bacterial counts in urine obtained by suprapubic needle aspiration and in mid stream urine specimens collected in connection with the puncture*

Organisms/ ml in the mid stream specimens	Organisms p r ml urine aspirated by suprapubic puncture Sterile	$<10^3$	10^3–10^4	10^4–10^5	10^5–10^6	$>10^6$
$<10^3$	27	1	1	0	0	0
10^3–10^4	17	1	5	0	0	0
10^4–10^5	0	0	2	3	1	0
10^5–10^6	2	0	0	1	6	1
10^6	0	0	0	0	1	26
Total no of pats	46	2	8	4	8	27

Even though none of the patients was heavily hydrated or had received any antibacterial agent prior to the needle aspiration fewer than 10 organisms per ml were found in 29 % (14/49) of the patients with bacteriuria From 12 of the 14 patients with fewer than 10 in the aspirated urine another mid stream specimen collected during a urine concentration test after admission to the hospital contained more than 10 The two remaining patients probably attained sterile urine spontaneously

DISCUSSION

In patients with urinary tract infection a considerable multiplication of bacteria takes place in the urine in the urinary tract (5 13) The number of bacteria in clean voided specimens from these patients usually far exceeds the number of bacteria due to contamination from the urethra and the periurethral region (6 12 14) In clinical practice quantitative urine culture is therefore used to

Table II *Per cent of successively collected mid stream specimens containing more than 10^5 or fewer than 10^4 bacteria per ml in relation to the number of bacteria found in the first specimen (I) and in the first two specimens with concordant counts (II)*

No of bacteria per ml in first specimen (I) and in the first two sp cimens (II)		$<10^3$	10^3–10^4	10 –10^5	10^5 10^6	10^6	Total no of specimens examined
Per cent of specimens with	I	6	7	34	6[illegible]	95	458
more than 10^3 organisms/ml	II	0	6	71	89	93	217
Per cent of specimens with	I	90	88	34	24	3	458
fewer than 10^4 organisms/ml	II	100	94	29	11	3	_17

distinguish between bacteriuria and contamination

In the majority of their patients with symptoms and signs of urinary tract infection Marple (8) and Kass (6) found more than 10 organisms per ml in catheter and clean voided urine specimens Stamey et al (13) found fewer than 10 in 33% of the specimens aspirated by suprapubic puncture from patients with bacteriuria and Goldberg et al (3) found fewer than 10 in 45% of the specimens in a similar study In both of these studies the puncture was performed after a period of heavy hydration which may have caused a considerable decrease of the bacterial count (3 11) In the present study needle aspiration was done without preceding hydration and fewer than 10 organisms per ml were found in 29 of the patients with bacteriuria

Several authors have found that the number of bacteria in clean voided specimens from women with no symptoms of urinary tract infection rarely exceeds 10^4 per ml (6 12 14) In a study involving 54 women whose urine was sterile when obtained by needle aspiration Stamey et al (13) found however that mid stream specimens collected in connection with the puncture contained 10^4 to 10 bacteria per ml in 11% and more than 10 in 7% of these cases Beard et al (1) found that mid stream specimens from 68 puerperal patients with sterile urine contained more than 10^4 in 12 cases In the present study more than 10 bacteria per ml were found in 4% of the mid stream specimens collected from 46 women whose urine was sterile

It must therefore be concluded that with regard to the number of organisms no definite boundary exists between contamination and significant bacteriuria in women The problem is to find out whether the results of quantitative urine cultures of one or more clean voided specimens indicate the presence or absence of bacteriuria with a degree of certainty sufficient for clinical practice

By comparing two bacterial counts of urine obtained from the same patient at different times Kass (7) found that the second specimen contained more than 10^5 in less than 2 % of the cases when the number of bacteria in the first specimen was below 10^4 In the present study culture of successive specimens revealed bacteriuria in 3 of the women with fewer than 10^4 in the first specimen Bacteriuria was however demonstrated by culture of urine obtained by needle aspiration in 4 of 42 women with fewer than 10^4 organisms per ml in two consecutive mid stream specimens In most of the cases of bacteriuria with low bacterial counts a third count on a concentrated urine specimen exceeded 10

In clinical practice bacteriuria may therefore be excluded if a single concentrated clean voided specimen contains fewer than 10^4 bacteria per ml

Kass (7) found that when the bacterial count of a cleanvoided specimen was in the range of 10^5 to 10^6 per ml a second specimen contained more than 10 organisms (Gram negative rods) per ml in only 67% of the cases In the present investigation 62% of such patients had more than 10 in a second specimen It must be stressed that 10 to 10^6 in a single clean voided specimen from a woman do not indicate bacteriuria with sufficient certainty

The presence of 10 or more bacteria per ml in two consecutive mid stream specimens or more than 10 in a single specimen increases the probability of bacteriuria to approximately 95%

Discrepancy between two bacterial counts or counts in the range of 10^4 to 10 does not permit any conclusion Either the examination should be repeated on a concentrated urine specimen or suprapubic needle aspiration should be performed

However it must be kept in mind that the diagnosis of bacteriuria based on the results of quantitative cultures of clean voided specimens rests upon the application of statistical probabilities to individual urinary findings (10)

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Acta med scand Vol 186 pp 39-45 1969

STUDIES IN URINARY TRACT INFECTIONS

II *Urinary Tract Infection Due to Coagulase negative Staphylococci*

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Abst act Coagulase negative staphylococci were found in the urine of 14% of 193 non pregnant women between the ages of 16 and 65 with significant bacteriuria. The leucocyte excretion rate in the urine of these patients was increased and was found to be of the same magni tude as in cases of infection due to bacteria known to be pathogenic in the urinary tract Pyuria disappeared as the patients obta ned sterile urine It is concluded that the patients were infected with coagulase negative staphylo cocci in the urinary tract

The majority of the patients had a depression of renal concentration capacity There was good agreement be tween the concentration capacity and clinical symptoms normally regarded as indicative of pyelonephritis It seems probable that coagulase negative staphylococci may cause pyelonephritis

Coagulase negative staphylococci were more frequently found in younger patients with urinary tract infection and were more frequent among patients without previous symptoms than among those with a history of recurrent urinary tract infection.

In comparison with patients with bacteriuria with E coli patients with coagulase negative staphylococci ob tained spontaneously sterile urine more frequently and more rapidly

Within the last few years coagulase negative staphylococci have been demonstrated in urine aspirated by suprapubic bladder puncture (14 21 23) in urine obtained from the ureter (3) and the renal pelvis (18) and by the culturing of renal biopsies (1 18)

It is often considered questionable whether coagulase negative staphylococci are pathogenic in the urinary tract A study has therefore been undertaken of the symptoms and the results of urine and renal examination of women with bac teriuria with coagulase negative staphylococci

MATERIAL

Since O tober 1966 general practitioners in the Roskilde area have been inv ted to refer all non pregnant women between the ages of 16 and 65 with symptoms or signs of urinary tract infection to the out patient clinic of Roskilde County Hospital

Most of the patients have been referred because of acute symptoms of urinary tract infection others have come due to unexplained symptoms such as tiredness backache or headache A few patients with asymptomatic urinary tract infection have been referred by doctors because routine examination revealed pyuria, hematuria or proteinuria.

As of April 1968 416 women had been examined at the hospital Significant bacteriuria was found in 193 patients all of these were admitted to the medical depart ment for further investigation

METHODS

All the women were interviewed by the author and came to the hospital on two consecutive days for urine ex amination The specimens were collected by a practised nurse using the mid stream technique described by Vejlsgaard (5) From February 1967 suprapubic bladder puncture was attempted on all new patients who had not urinated during the last two hours before arrival.

Quantitative bacterial culture of the urine was per formed using the calibrated loop technique on nutrient agar with lactose on blood agar plates, and by streaking of the inoculum with a sterile glass rod (6) Bacteriuria was diagnosed by isolation of bacteria from urine ob tained by suprapubic puncture or demonstration of sig nificant bacteriuria, i e more than 10⁵ bacteria in two consecutive mid stream specimens (7 13)

Identif cation of the isolated bacteria was undertaken by the Department of Diagnostic Bacteriology Statens Seruminstitut Copenhagen. B ochemical characteristics of some of the isolated coagulase negative staphylococci have been published by Mortensen (17)

A method for determination of the leucocyte excret on rate in urine has previously been descr bed (1?) and the excret on in normal persons has been found to be less than 400 000 leucocytes per hour W th the technique employed for microscopic exam nation of urinary sedi ment it has been shown that three or more leucocytes per HPF indicate pyuria (1) Hematuria was d agnosed by demonstrating three or more erythrocytes per HPF or by def nitely posit e benzidine reaction in the urine

The renal concentration capacity was estimated by

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eıther 0 5 g of sulphamethızole or 0 75 g of ampıcıllın for 14 days A thırd of the patıents were treated with placebo usıng the double blınd technıque The tablets were dıstrıbuted ın numbered bottles every thırd bottle contaınıng placebo tablets

RESULTS

Of the 193 non pregnant women between the ages of 16 and 65 wıth sıgnıfıcant bacterıurıa 27 had bacterıurıa wıth coagulase negatıve staphylococcı (Table I) Twenty two of these had more than 10^6 organısms per ml ın at least two consecutıve urıne specımens Two had more than 10^6 ın one specımen and more than 10 ın the other and one had between 10 and 10^6 ın both specımens In the remaınıng two cases where there were fewer than 10 bacterıa per ml the dıagnosıs was confırmed by culture of urıne aspırated by suprapubıc puncture One addıtıonal patıent was found to have a mıxed ınfectıon wıth E colı and coagulase negatıve staphylococcı The dıagnosıs was confırmed by bladder puncture thıs patıent ıs not however ıncluded ın the materıal under consıderatıon here

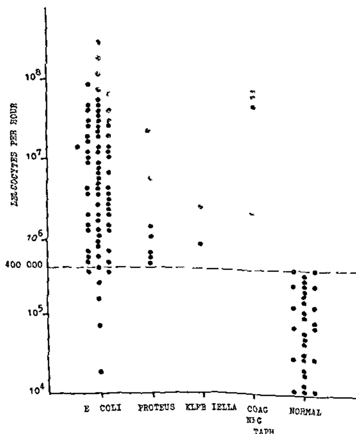


Fig 1 Hourly leucocyte excretıon ın the urıne ın 32 normal women and ın 1?? women wıth bacterıurıa The latter group ıs subdıvıded accordın organısm ısolated

The leucocyte excretion rate for the last 20 patients was determined after admission to hospital The leucocyte excretion was found to be of the same magnitude as in infections due to bacteria known to be pathogenic in the urinary tract (Fig 1)

A comparison of the results of microscopy of urine sediment in the presence of bacteriuria and the findings 14 days after the urine had become sterile was made to determine further the correlation between pyuria and bacteriuria with coagulase negative staphylococci Of the 27 patients examined 22 had three or more leucocytes per HPF in the first specimen and 26 in at least one specimen before the urine became sterile No patient had more than two leucocytes per HPF after two weeks with sterile urine

Among all patients 34% had hematuria and 47% had urinary protein concentrations exceeding 0 25 p p m Forty four per cent of the patients with coagulase negative staphylococci had hematuria and more than 0 25 p p m of protein was found in 44 of specimens from these patients

Urinary tract infection due to coagulase negative staphylococci was most frequent among the younger patients (Table II) Twenty three % of the patients between 16 and 25 were infected with coagulase negative staphylococci while only 8 5% of the patients over 25 had coagulase negative staphylococci in the urine ($\chi^2 = 7\ 670$ $p < 0\ 01$)

All the patients have been under continued observation and by April 1968 there had been 130 instances of significant bacteriuria after a period with sterile urine 46% of which were reinfections with coagulase negative staphylococci Urinary tract infections due to coagulase negative staphylococci were found to be considerably less frequent among the reinfected patients than in the original material of 193 patients ($\chi^2 = 7\ 48$ $p < 0\ 01$)

Coagulase negative staphylococci were found more frequently in patients without previous symptoms of urinary tract infection than in patients with previous symptoms (Table III) No patients with asymptomatic urinary tract infection had coagulase negative staphylococci in the urine A relatively large number of the patients with coagulase negative staphylococci had back ache and three pa[illegible] clinically acute pyelonephritis with fever and flank pain (Table IV) The duration of symptoms before arrival at the clinic was approximately the same among patients with coagulase negative staphylococci and in the material as a whole (Table V)

The serum creatinine concentration did not exceed 1 1 mg per 100 ml for any of the patients Intravenous urography was performed on all patients one patient showed slight signs of pyelone

Table II *Age distribution*

Age y	16– 5	26–35	36–45	46–55	56–65
All patients	39	20	19	9	13
Patients with coag neg staph	63	7	7	11	11

Table III *Previous symptoms*

I No previous symptoms of urinary tract infection II One to three episodes of dysuria and frequency III More than three episodes of dysuria and frequency IV Previous symptoms of pyelonephritis with fever and flank pain associated with symptoms from the lower urinary tract

	I	II	III	IV
All patients	28	26	19	27
Patients with coag neg staph	44	22	15	19

Table IV *Subjective symptoms upon arrival at the hospital*

I Dysuria and frequency II Dysuria frequency and back ache III Backache only IV Flank pain and fever V No subjective symptoms

	I	II	III	IV	V
All patients	48	21	5	11	15
Patients with coag neg staph	48	41	0	11	0

Table V *Duration of symptoms before first examination*

Duration (days)	1–3	4–10	11 30	>30	No symptoms
All patients	27	29	2[illegible]	8	15
Patients with coag neg. staph	37	33	27	4	0

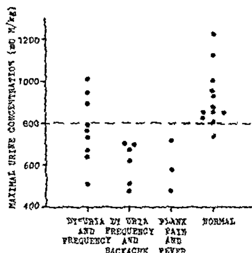


Fig 2 Maximal urine concentration capacity in relation to clinical symptoms in 20 patients with urinary tract infection due to coagulase negative staphylococci The figure shows in addition the concentration capacity in 15 convalescents without signs or symptoms of urinary tract disease

phritis and one patient had a dilatation of one ureter with no indication of obstruction No definitely abnormal condition was discovered in any the other patients

The maximum urine concentration capacity as determined for the last 20 patients The results are shown in Fig 2 together with the concentration capacity in 15 convalescents without present or previous symptoms of urinary tract disease Seventeen of the patients with urinary tract infection due to coagulase negative staphylococci had a maximum urine concentration capacity of less than 800 mOsm per kg There was good correlation between symptoms normally considered indicative of pyelonephritis and the concentration capacity (Fig 2) Some correlation was found between subjective symptoms and protein concentration in the urine (Table VI)

In five cases the serum was tested for antibodies against the isolated coagulase negative staphylococci upon arrival at the clinic and 14 days later A definite rise in titre was found in only one case An inhibition test showed that the antibodies were neutralized by the strain of coagulase negative staphylococci isolated from the patient However this patient had no other signs of acute pyelonephritis and other patients with clinically acute pyelonephritis had no antibody titre rise

Table VI *Symptoms and urinary protein concentration in patients with coagulase negative staphylococcal bacteriuria*

I Dysuria and frequency II Dysuria frequency and backache III Flank pain and fever

	I	II	III
Protein concentration			
Less than 1/4 ‰	9	6	0
1/4 ‰ to 1 ‰	4	5	1
1 ‰ or more	0	0	2

All patients treated with sulfamethizole or ampicillin had sterile urine within one week after the start of treatment After completion of treatment one patient relapsed with coagulase negative staphylococci after a period with sterile urine

A third of the patients nine in all were treated with placebo In the course of two months eight of these obtained sterile urine spontaneously One patient had bacteriuria with coagulase negative staphylococci for a period of eight months without any subjective symptoms At this time she became pregnant and was treated In all the placebo-treated patients who had bacteriuria for more than one week the subjective symptoms disappeared before the urine became sterile The proportion of patients with sterile urine in relation to all the placebo treated patients during the observation period is shown in Fig 3 together with the course of 31 placebo treated patients with bacteriuria with E coli

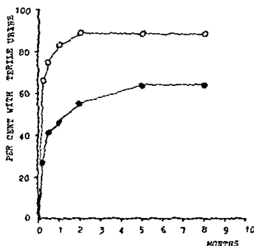


Fig 3 Per cent of placebo treated patients with sterile urine in the observation period O—O patients with coagulase negative staphylococci ●—● patients with E coli bacteriuria

DISCUSSION

Coagulase negative staphylococci are usually considered harmless saprophytes Through investigation of the bacterial flora in the female urethra Cox (2) found staphylococcus albus to be the organism most frequently isolated This probably explains the fact that this is a frequent contaminant in specimens obtained via the urethra (11 22) The demonstration of coagulase negative staphylococci in the urine has therefore been regarded with scepticism

Within the last few years several authors (14 21 23) have found coagulase negative staphylococci in urine aspirated by suprapubic bladder puncture Coagulase negative staphylococci have also been found in urine obtained from the ureter (3) and from the renal pelvis (18) and by culture from renal biopsies (1 18)

It is often considered questionable whether coagulase negative staphylococci are pathogenic in the urinary tract The author therefore examined 27 women with significant bacteriuria with coagulase negative staphylococci for other signs and symptoms of urinary tract infection

Pereira (20) Gallagher et al (5) and Mitchell (16) state that most of their patients with coagulase negative staphylococci in the urine had pyuria as determined by microscopy of urine sediment

In the present study the leucocyte excretion rate was determined and found to be of the same magnitude as in infections due to bacteria known to be pathogenic in the urinary tract This confirmed that the patients had inflammatory disorders of the urinary tract (4) Judging by the results of examinations of urine sediment the pyuria disappeared in all patients within two weeks after the urine had become sterile It must therefore be concluded that the patients were infected with coagulase negative staphylococci in the urinary tract

The concentration capacity was reduced in most patients in the present study and there was good agreement between subjective symptoms of pyelonephritis and depression of renal concentration capacity Low concentration capacity does not in all cases however indicate a diagnosis of acute pyelonephritis Some patients may have reduced concentration capacity due to irreversible chronic pyelonephritis The patients are therefore under continued observation for possible changes in renal concentration capacity (26) On the basis of the information available it must be assumed that coagulase negative staphylococci may cause pyelonephritis

The serum of some patients was examined for antibodies against the isolated strains of coagulase negative staphylococci This test was found to be of no value in the differential diagnosis between pyelonephritis and infection of the lower urinary tract

Many authors have rejected coagulase negative staphylococci found in the urine regarding them as contaminants or as apathogenic This makes it difficult to obtain an impression of the frequency of urinary tract infection due to coagulase negative staphylococci

Through systematic investigation in a general practice of all patients with symptoms of urinary tract infection Gallagher et al (5) found 88 patients with significant bacteriuria Fourteen % of them were infected with coagulase negative staphylococci In a similar investigation Steensberg et al (24) found significant bacteriuria in 223 mostly younger women Coagulase negative staphylococci were isolated from 14% of the total number In the same investigation bacteriuria was found in 34 males only one of them had coagulase negative staphylococci in the urine

McFayden and Eykyn (14) performed suprapubic bladder puncture on 1000 pregnant women and found 59 with bacteriuria 11 of these had coagulase negative staphylococci In a screening study of pregnant women Kincaid Smith (8) found coagulase negative staphylococci in 14 of the patients with significant bacteriuria while Little (10) in a similar investigation found this organism in only 1% of all patients with urinary tract infection

In a general medical department Kleeman et al (9) found coagulase negative staphylococci in 1 of males and pregnant and non pregnant women with significant bacteriuria Mitchell (16) reported that coagulase negative staphylococci were frequently found in bacteriuria following instrumentation of the urinary tract

Among 193 non pregnant wom n between the age of 16 and 65 with significant bacteriuria coagulase negative staphylococci were found in 14 predominantly among the younger patients It must be stressed that all of them were patients who normally would have been treated by their family doctors and not referred to hospital

All the patients are under continued observation so far 130 reinfections have occurred in only 4 8% of these reinfections have coagulase negative staphylococci been found This suggests that coagulase negative staphylococci occur less frequently in patients who have recurrent urinary tract infection This is in accordance with the fact that bacteriuria with coagulase negative staphylococci is less common among hospital patients with chronic pyelonephritis who have been admitted because of recurrent or complicated urinary tract infection The patients who had recurrences during the observation period cannot be compared without reservation with the original patient material because they differ from them in respect of age distribution and frequency of previous urinary infection

In comparison with the rest of the patients those with coagulase negative staphylococci had fewer previous periods with symptoms of urinary tract infection However this relationship is of little significance because there is some correlation between age and frequency of previous infection

Asymptomatic urinary tract infection may be o scantily represented among the patients studied as most of them sought their family doctors because of symptoms of urinary tract infection However it must be noted that among 27 patients with asymptomatic bacteriuria there were none with coagulase negative staphylococci in the urine

A comparison of the duration of symptoms before the first examination at the hospital of patients with coagulase negative staphylococci in the urine and the duration of symptoms among all the patients showed no differences There was no indication that the first group sought a doctor sooner or later than the other The discomfort suffered by both groups is apparently equally pronounced

All the patients treated with chemotherapeutics or antibiotics obtained sterile urine within a week Nine patients were treated with placebo and eight of these attained spontaneously sterile urine in the course of two months Compared with the course of patients infected with E coli patients with coagulase negative staphylococci attained sterile urine more frequently and more rapidly The groups are not however entirely comparable with regard to age and frequency of previous urinary tract infection as selection was based on the type of organism isolated from the urine

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Acta med scand Vol 186 pp 47–51 1969

STUDIES IN URINARY TRACT INFECTIONS

III *Biochemical Characteristics of Coagulase negative Staphylococci Associated with Urinary Tract Infections*

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Abstract Forty six strains of coagulase negative staphylococci from 13 patients with established urinary tract infections have been examined by biochemical tests None of the strains produced coagulase or fermented mannitol The strains differed in their production of haemolysins and phosphatase phage sensitivity liquefaction of gelatin reduction of nitrate splitting of Tween 80 pigmentation and production of acid from glucose mannitol and lactose

It is concluded that contrary to prevailing opinion a negative result in the coagulase test on staphylococci isolated from urine does not exclude their association with infections of the urinary tract

Although generally disregarded as agents of disease coagulase negative staphylococci have been isolated from urine under circumstances which point to their role as pathogens by Pereira (14) Gallagher et al (4) Roberts (15) Mitchell (9) Mabeck (8) and others

Roberts (15) and Mitchell (9) identified the staphylococci isolated from the urinary tract by use of the key devised by Baird Parker (1)

The present paper deals with the biochemical characteristics of 46 strains of coagulase negative staphylococci isolated from 13 female patients with urinary infections

MATERIAL AND METHODS

Forty-six strains of coagulase negative staphylococci from 13 patients with urinary infections were available for examination The strains were isolated by Mabeck during the last part of his study when the possible pathogenic role of coagulase negative staphylococci in infections of the urinary tract was recognized The association of these strains with urinary infection is based upon the criteria described by Mabeck (7) all of which were fulfilled All 46 strains were isolated on different occasions from mid stream urine and in some cases, from urine obtained by suprapubic aspiration of the bladder

Morphology Gram staining, motility arginine decomposition, production of catalase coagulase deoxyribonuclease oxidase phosphatase and urease (method b only) gelatin hydrolysis, H_2S formation reduction of nitrate splitting of Tween 80 and the Voges-Proskauer reaction were performed and read all according to the methods described by Mortensen and Kocur (10)

Production of acid from carbohydrates pH of cultures grown aerobically and anaerobically in a fluid medium containing glucose was determined as described by Mortensen and Kocur (10) The semisolid Standard Medium of the Subcommittee on Taxonomy of Staphylococci and Micrococci (20) and the medium of Hugh and Leifson (5) were used with and without a paraffin oil cover for measurement of production of acid from glucose and mannitol The Hugh and Leifson medium was used without a top layer of paraffin oil for the determination of acid production from arabinose galactose maltose and lactose

Production of β galactosidase was examined by use of an o-nitrophenyl β D-galactoside (ONPG) test in the modification of Bülow (3)

Gelatin liquefaction was also studied by Kohn's method as modified by Lautrop (6) a positive result by either method is recorded as positive

Haemolytic activity was assessed by placing a loopful of a 5–6 hour broth culture on nutrient agar plates with 5% defibrinated rabbit and sheep blood and subsequently incubating at 35 C for 18 hours and then at 4 C for 24 hours

Novobiocin sensitivity was examined by the prediffusion method of Thomsen (21) using a medium of beef heart broth containing agar approximately 1 8% defibrinated horse blood, 10 glucose 1% sodium chloride 0 3% Na_2HPO_4 12 H_2O 0 ?% Paper discs impregnated with 25 μg of sodium novobiocin (Antibiotic Dep Statens Seruminstitut) were applied to the plates 18 hours before inoculation By this method an inhibition zone with a diameter of more than 25 mm corresponds to a [illegible] inhibitory concentration of less than 2 μg ml and indicates sensitivity The novobiocin sensitivity test

Table I *Production of acid from glucose in different media*

	Type of fermentation	pH in fluid medium		Standard medium		Hugh & Leifson medium	
		Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic
	A	4 3–4 8	4 3–4 6	+	+	+	+
	B	5 2–5 8	4 9–5 3			+	+
Ino ulated control tubes without glucose		6 8–7 2	6 9–7 9	–	–	–	–
Uninoculated tubes with and without glucose		6 8–7 0	6 9–7 2	–	–	–	–

+ = positive = negative = weak reaction in the top part of the medium

Range of values after incubation for 2 4 or 6 days

was also performed with 5 sensitive and 5 resistant strains on the above medium without addition of glu ose

Phage typing was done by the method of Blair and Williams (2) using the international basic set of typing phages supplemented as described by Rosendal et al (16) and by Rosendal and Bulow (17) All strains were ex amined using both the routine test dilution (RTD) and the dilution RTD x 1000

RESULTS

Common characteristics

All strains were found to be non motile Gram positive cocci growing in pairs and clusters All strains gave positive results in the tests for pro duction of catalase urease and acid from galac tose and maltose They gave negative results in the tests for production of coagulase deoxyri bonuclease H S oxidase and acid from arabinose No strains fermented mannitol by the criterion and method of the Subcommittee (19 20) In the Vogus Proskauer test only some of the strains gave po itive results after an incubation period of two days but all were positive when the test was done after incubation for five days All strains produced a reaction in the arginine test when this was performed with suspensions of bacteria

Differential characteristics

The strains could be divided into two types (A and B) according to differences in their produc tion of acid from glucose This was shown by the pH changes taking place in fluid medium and by the colour changes occurring in the Standard medium (Table I)

Type A was characterized by pronounced and rapid formation of acid both aerobically and anaerobically with pH values ranging from 4 3 to 4 8 from the second day of incubation and by positive results in the Standard medium by the criterion of the Subcommittee (19) Type B also produced acid from glucose under anaerobic and aerobic conditions but did so less markedly pH values of the anaerobic cultures were in the range 5 2 to 5 8 In the Standard medium negative re sults were recorded although colour changes to yellow were observed in the upper part of the medium In the medium of Hugh and Leifson all strains of both types A and B gave positive results

Table II shows a division of the strains into biotypes taking into account all differences ob served The results of phage typing are listed in Table III The variations in phage susceptibility among the strains from patient IM are considered too small to indicate distinct populations With two exceptions strains isolated from the same patient belonged to the same biotype One strain from patient EN differed from three other strains from this patient in three properties viz pigmen tation phage sensitivity and ability to liquefy gelatin The strains from patient MH belonged to two different biotypes but these were isolated at an interval of over six months

Strains having glucose fermentation of type A differed from type B strains in several other respects they gave positive results in the arginine reaction by the method of Møller (13) produced phosphatase reduced nitrate split Tween 80 and were sensitive to novobiocin but did not produce acid from mannitol in either medium The strains having glucose fermentation of type B displayed various patterns of behaviour and differed in their production of acid from lactose phage sen

Table II *Differences between the strains*

Pat	No of strains	Type of glucose ferment	Arginine reaction	Haem of sheep red cells	Nitrate to nitrite	Novobiocin sens	Phosphatase	Tween 80	Mannitol: Stand medium	Mannitol: H & L medium	Lactose: H & L medium		Pigment	Gelatin	Phage typing
									An & ae	An & ae	Ae	ONPG			
VC	3	A	+	+	+	S	+	+	–	–	+	+	–	+	NT
NT	4														
UH	2														
BMA	1														
MN	2	A	+	–	+	S	+	+	–	–	+	+	–	+	NT
IM	3	B	X	–	–	R	–	–		+	–	–	Orange	–	+
EN	3	B	X	–	–	R	–	–		+	+	+	Orange	–	+
EN	1	B	X	–	–	R	–	–		+	+	+	–	+	NT
SP	11														
LC	2	B	X	–	–	R	–	–		+	+	+	Orange	–	NT
MH	3														
MH	3	B	X	–	–	R	–	–		+	–	–	Orange	+	NT
KH	3	B	X	–	–	R	–	–		+	+	+	Orange	+	NT
LH	3	B	X	–	–	R	–	–		+	+	+	Cream	+	NT
KJ	2	B	X	–	–	R	–	–	–	+	+	+	–	–	NT

+ = positive   – = weak reaction in the top part of the medium   – = negative   X = positive in rapid test, negative in growth test
S = sensitive   R = resistant   An = anaerobically   ae = aerobically   NT = not typable

See Table III

Table III *Results of phage typing*

Pat	Strains	Phage type / pattern	
		RTD	RTD × 1000
	6148	NT	83A (B)
EN	6149	NT	83A (B)
	6177	NT	83A (B)
	6498	NT (77/85 + w)	3A/3C/55 6/42E/47/54/77/81/83A/85
IM	6499	77/85 +	52/52A/79/80 3A/3C/55/6 42E/47/53/54/75/77/81/83A/84/85/42D 6557/
	6500	NT	3A/3C/55/6/42E/47/54/75/77/81/83A/85 +

RTD = routine test dilution NT = not typable

sitivity pigmentation and ability to liquefy gelatin Haemolytic activity was recorded only in some of the strains of type A

The glucose content of the medium in the novobiocin sensitivity test did not influence the division of strains into sensitive or resistant types but the inhibition zone produced by the resistant strains decreased from 9–12 mm to ≤ 6 mm in the medium without glucose

DISCUSSION

Roberts (15) isolated Gram positive catalase positive cocci from suprapubic urine specimens in 20 patients By Baird Parker s key 14 were Micrococcus and six Staphylococcus strains Eleven of the Micrococcus strains were of sub group 3 two of subgroup 2 and one of subgroup 7 All strains of the Staphylococcus group belonged to subgroup II Mitchell (9) reported that strains likely to have caused infection belonged to Baird Parker s Micrococcus subgroup 3 in 39 cases which corresponded to about a quarter of all the cases he examined the remainder belonging to the Staphylococcus subgroups II (72 patients) IV (4 patients) V (21 patients) and VI (11 patients) Thirty eight of the 39 cases of infection with Micrococcus subgroup 3 had acquired their infec tion outside hospital

The findings reported in this paper confirm the occurrence of coagulase negative staphylococci in patients from domiciliary practice with infection of the urinary tract and the existence of different levels of glucose fermentation capacity among such strains The strains isolated from 8 of 13 patients could be identified as Micrococcus by use of the scheme of Baird Parker and by their production of acetoin they could be placed in one of his subgroups 1 2 3 or 4 However dif ferences in the composition of the media used to detect acid produced from carbohydrates do not permit a definitive subgrouping The strains iso lated from the remaining 5 of the 13 patients gave similar reactions to those of his Staphylococcus subgroup II

The proportion of strains fermenting glucose rapidly under anaerobic conditions differs from the frequency reported earlier by Mortensen and Kocur (11) where 269 of 285 clinical isolates exhibited such type A fermentation However no isolates from urine were included in that study (12)

The positive results of phage typing are in ac cordance with the results of Spink and Strong (18) who found 16 strains of coagulase negative staphylococci phage typable among 485 clinical isolates

The present work emphasizes that the results of a coagulase test should not be used indiscrimi nately as an indication of the pathogenicity of staphylococcal agents of infections of the urinary tract Whether this holds true also for strains associated with other infections remains to be investigated more fully

Furthermore the observations here reported on strains having an established association with in fection show that pathogenicity cannot be ex cluded by the properties in which the strains dif fer (e g production of phosphatase and haemo lysins sensitivity to novobiocin liquefaction of gelatin pigmentation reduction of nitrate split ting of Tween 80 and production of acid from glucose mannitol and lactose)

Therefore before clinical conclusions are drawn

from a laboratory report of isolation from urine of staphylococci whether coagulase positive or negative account should be taken of such other factors as the symptoms of the patient the conditions of sampling the number of bacteria per ml urine the excretion of leucocytes etc

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HEMODYNAMIC EFFECTS OF LIDOCAINE

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Abstract Lidocaine is currently one of the most used drugs in ventricular arrhythmias, especially in acute myocardial infarction. Only few series subjected to hemodynamic studies have hitherto been reported The author studied the hemodynamic changes following the intravenous injection of lidocaine to 12 cases with non ischemic heart disease After a bolus dose of 50 or 100 mg an infusion was started with 1 or 2 mg/min for 20 minutes. No significant changes were found in mean values for heart rate cardiac output, stroke volume mean systemic and pulmonary artery pressures or peripheral arterial resistance It was well tolerated in all cases, including one with total A V block The serum lidocaine concentration was determined in eight cases.

After some earlier animal experiments (3) the first successful conversion of ventricular fibrillation in man was performed in 1942 with the aid of procaine (1) Though effective procaine has a depressant effect on blood pressure (15) Lidocaine (lignocaine Xylocaine®) was synthesized in Sweden in 1943 It has gained widespread use as a local anesthetic but has also a place in the treatment of status epilepticus

Lidocaine was first tried in ventricular fibrillation in 1950 by Southworth et al (19) In that case countershock was effective only after the intracardial administration of lidocaine However procaine had been given previously though apparently without effect Further lidocaine was given in a preparation with adrenaline 1 100 000 and in small doses Thus it seems difficult to ascribe the effect in this case to one drug

Since 1953 a number of papers have appeared on the use of lidocaine in experimental animals Since about 1959 it has gained increasing clinical use as an antiarrhythmic agent During the last years lidocaine has in many coronary care units including our own been the method o choice for the treatment of ventricular ectopic bea s

Since only few studies have been reported concerning the hemodynamic effects of lidocaine the present work was started. The main question has been the effects on heart rate cardiac output and systemic arterial pressure in adult cardiac cases with different degrees of disability

MATERIAL

Twel e cases between 24 and 61 years were referred for routine diagnostic heart catheterization Their diagnosis are listed in Table I They were all in function classes II–III Sinus rhythm was present in eight cases, atrial fibrillation in three and total A V block in one case All cases were compensated at the time of the in estigation

METHODS

Right heart catheterization was performed in all cases, with percutaneous insertion of a polythene catheter in the brachial artery In two cases a transseptal left heart catheter was also inserted. Only local anesthesia was used. After insertion of the catheters the patient rested for 10 min Cardiac output was then determined using Fick s direct method Expired air was collected during 10 min and the oxygen consumption was determined by Haldane s method Arterial and mixed venous blood gases were analyzed by a spectrophotometric method

Lidocaine was then injected ia the right heart catheter A bolus dose of 50 or 100 mg was injected during - min, immediately followed by a drip of 1 or mg min for 18 min During the last 10 min cardiac output was measured Thus a total of 70–140 mg was given during 0 min The serum concentration of lidocaine was determined in eight cases by means of gas chromatography The samples were taken 15 min after the injection.

RESULTS

These are pres nted in Table I Irrespective of the doses given there were no statistically significant differences in mean values for cardiac output h art rate stroke volume or systemic arterial pres

Table I *Hemodynamic changes following lidocaine administration*

Case no	Age (y)	Sex	Diagnosis	Rhythm	Lidoc dose	Lidoc conc. (μg/ml)	CO (l/min) B	CO (l/min) A	HR B	HR A	SV (ml) B	SV (ml) A	Systemic arterial pressure (mm Hg) B	Systemic arterial pressure (mm Hg) A
1	53	♀	AS+AI	A-V block	50+1	0.9	5.6	4.8	41	39	136	123	138/64 85	149/62 95
2	61	♀	Stat. p myoc.	Sinus	100+2	1.5	4.3	4.6	60	61	72	76	133/80 112	140/8[illegible] 105
3	24	♂	ASD op	Sinus	50+2	2.0	5.8	5.0	52	52	112	96	144/81 113	140/80 110
4	60	♀	MS+AI	Atr fibr	50+1	2.6	4.4	4.0	6[illegible]	61	72	66	1[illegible]8/72 97	1[illegible]4/70 90
5	53	♀	Dolor pect	Sinus	50+1	5.2	4.4	4.9	53	54	83	91	132/80 100	135/80 100
6	50	♀	MS	Atr fibr	50+1	1.[illegible]	3.7	3.3	89	89	4[illegible]	36	134/70 104	130/80 98
7	44	♂	MS	Sinus	50+1	—	3.6	3.7	7[illegible]	70	50	53	1[illegible]5/70 90	125/70 90
8	44		MS+MI	Atr fibr	50+1	—	3.1	2.4	56	54	55	44	130/78 109	1[illegible]8/80 106
9	44		Stat p myoc	Sinus	50+1	—	5.3	6.4	72	73	74	88	122/70 90	118/70 90
10	37	♀	ASD+MI	Sinus	50+1	—	[illegible]0.7	17.3	75	76	271	[illegible]76	12[illegible]/68 85	1[illegible]0/70 85
11	41	♀	MS	Sinus	100+2	3.7	3.8	4.1	60	61	63	67	122/65 82	140/75 95
12	56		MS	Sinus	100+[illegible]	6.5	3.7	3.9	57	57	65	68	120/67 84	123/71 100

AS = aortic stenosis. AI = aortic insufficiency MS = mitral stenosis. ASD = atrial septal defect of secundum type Stat p myoc = status post myocarditis. CO = cardiac output HR = heart rate SV = stroke volume Pressures systolic/diastolic mean Case 10 CO and SV mean pulmonary blood flow and right ventricular stroke volume respectively
Dose in mg and mg/min respectively B = before A = after lidocaine

sure This applies also to case 1 who had a third degree A V block as well as to case 12 the only patient who experienced side-effects Immediately after the bolus injection, she complained of slight lightheadedness which disappeared during the following constant infusion The values for CO and SV in case 10 were not included in the statistical test The values for oxygen consumption, arterio venous oxygen difference mean pulmonary artery pressure and peripheral arterial resistance are not reported Nor were any statistically significant changes in these parameters found after lidocaine

The mean percentual changes were for cardiac output −3% (range −23% to +21%) for heart rate ±0% (range −5% to +2%) for stroke volume −3% (range −20% to +19%) for mean arterial pressure +2% (range −6% to +19%)

In cases 8 9 and 10 there were significant changes in cardiac output during xylocaine administration No untoward effects were noted nor any restlessness or anxiety as regards the procedure

Serum lidocaine concentrations varied between 0.9 and 6.5 μg/ml

DISCUSSION

Experimental investigations have shown that, in doses comparable to those used clinically lidocaine has no or only a slight depressant effect on cardiac output (5 14) though conflicting results have also been reported (2)

Only few series have hitherto been reported on its effects in patients Kimney and Steinhaus (15) and Harrison et al (11) compared the effect of lidocaine with that of procaine and procainamide respectively in cases under general anesthesia Recently some results have been published concerning the hemodynamic changes following lidocaine in patients without general anesthesia In cardiac patients without myocardial infarction no consistent changes in cardiovascular performance were noted after a single intravenous injection of 50–100 mg (18) nor when a loading dose of 1 mg/kg was followed by 1 mg/min (4) However though there were no significant changes in mean values signs of a slight myocardial depressant effect was found in four out of eight cases who received a dose of 100 mg These effects were of short duration and unaccompanied by any symptoms (16)

A few studies have been reported concerning the effects in cases with acute myocardial infarction (13 20 21) No significant changes were found in brachial artery pressures heart rate cardiac output or stroke volume after an intravenous injection of 1 mg/kg (13) or 100 mg (21)

These earlier results are confirmed by the present study in which a loading dose of 50–100 mg

was immediately followed by an infusion with 1–2 mg/min

The problems concerning dosage blood levels and toxicity have been analyzed (7 9 10 12 13) The therapeutic serum level is between 1 and 4 μg/ml This is usually obtained with an infusion rate of 25–35 μg/kg/min which in a 70 kg man will correspond to 1 75–2 45 mg/min Toxic effects have been noted at levels in excess of 6 μg/ml (6 10) As lidocaine is metabolized to a large extent in the liver great caution should be used when giving the drug to patients with impaired liver function With higher serum concentrations there may be twitchings or general convulsions It should be noted that immediately after a single injection of 1 mg/kg serum levels of about 10 μg/ml are recorded (13) This could explain the values found in the present study

It has been stated that lidocaine is contraindicated in patients with A V dissociation and a slow nodal or idioventricular pacemaker (8) In experiments on dogs with a surgically total A V block lidocaine was found to slow the ventricular rate (7 16) In the case reported here (case 1) no untoward effects were noted after lidocaine It has recently been found that lidocaine has no influence on the ventricular stimulation threshold in patients with total A V block and pacemaker (17) However lidocaine should be used with great caution in cases with second and third degree A V block unless a pacemaker is immediately available

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NON HYPERTENSIVE ANGIOPATHY

Renal Biopsy Study of the Nephrotic Syndrome

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Abstract The thickness of the wall of the small arteries and arterioles has been measured microscopically from renal biopsies of 36 normotensive patients forming three groups 1) 12 patients with lipoid nephrosis or glomerulonephritis with the nephrotic syndrome 2) 11 patients with glomerulonephritis without the nephrotic syndrome and 3) 13 patients with various unrelated diseases Groups 2 and 3 served as controls The patients with nephrosis showed thickening of the renal vessels The difference from both control groups was statistically significant. The renin angiotensin aldosterone system operating in secondary hyperaldosteronism is thought to be the cause of the vascular changes.

Hypertensive angiopathy in familial chloride diarrhoea a non hypertensive state was reported by Pasternack and Perheentupa (5) It was supposed that the vascular alterations thickening of the walls of small arteries were due to prolonged vasoconstriction caused by the high angiotensin activity related to the secondary hyperaldosteronism in this state Recently Brackett et al (3) reported a case of Bartter s syndrome having the same vascular alterations Bartter s syndrome includes some of the characteristics of familial chloride diarrhoea namely juxtaglomerular hyperplasia high renin activity high aldosterone excretion and absence of hypertension

Because of these observations we decided to study the arterioles and small arteries in kidney biopsies from non hypertensive patients with the nephrotic syndrome i e another state of secondary hyperaldosteronism

MATERIAL AND METHODS

The series consists of renal biopsies from 36 patients with normal blood pressure and unimpaired renal function

Twelve of the patients displayed the nephrotic syndrome In four of them only minimal glomerular changes were noted in the renal biopsy and the aetiology was considered to be lipoid nephrosis. In eight nephrotics the aetiology was chronic glomerulonephritis All patients of this group were heavily oedematous at the time of the biopsy Three of them had been treated with adrenocortical steroids maximally for three months

Eleven patients with chronic glomerulonephritis without nephrosis were studied as controls

Thirteen patients served as further control In these patients the renal biopsy was done for various reasons irrelevant to the present issue The morphological finding was normal

The renal biopsy specimens were fixed in 10 neutral formaldehyde embedded in paraffin wax, sectioned at 3-5 μ and stained with periodic acid Schiff (PAS) and silver methenamine according to Gomori.

From each patient/biopsy specimen about ten cross sections of small arteries and arterioles were measured with an eye piece micrometer under high power objective (400 x) For each vascular cross section a quotient was calculated from the vascular and luminal diameter The more thickened the vessel the greater the quotient

For statistical analysis the mean quotient for each patient was calculated The mean values of the nephrotic patients were statistically tested against the mean values of the patients of both control groups Correction for small samples was performed ( )

RESULTS

The results are given in Figs 1-4 In Figs 1-3 the quotients of each vessel in the nephrotic and control groups are plotted in relation to the age of the patients The figures show that there was a greater spread of the quotient values in the nephrotic patients than in the controls In the nephrotic group there were 17 123 quotient values over 3 0 compared with 6 241 in the control series In this series no correlation could be seen between the quotient values and the age of the

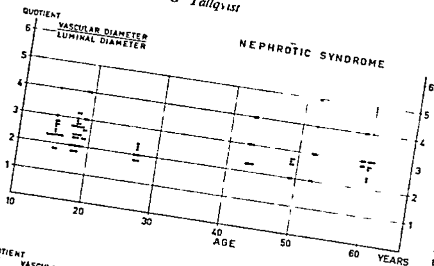


Fig 1 Quotient values (vascular diameter/luminal diameter) of small arteries and arterioles in relation to patient s age Vessels from kidney biopsies of normotensive patients with nephrotic syndrome

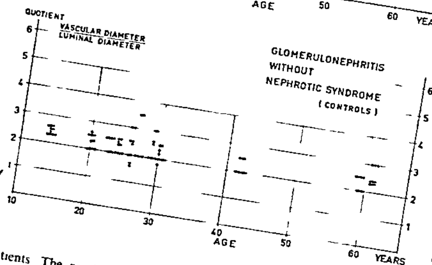


Fig Quotient values (vascular diameter/luminal diameter) of small arteries and arterioles in relation to patient s age Vessels from kidney biopsies of patients with glomerulonephritis absence of nephrotic syndrome and hypertension

patients The results imply that there are vessels of both normal and abnormal thickness in the nephrotic group

Fig 4 gives the mean quotient values of the patients in the different groups and the mean of each group The difference of the mean values of the nephrotic group and the control groups is statistically significant (nephrotic syndrome/glomerulonephritis $P < 0\ 001$ nephrotic syndrome/other controls $0\ 01 > P > 0\ 001$)

The thickening of the vessels was mostly due to cellular hyperplasia In a few cases the arteriolar wall showed strong PAS positive staining in some areas of the intima and media The two changes occurred side by side in the same patients (Figs 5–7)

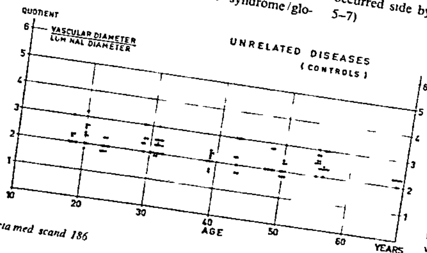


Fig 3 Quotient values (vascular diameter luminal diameter) of small arteries and arterioles in relation to patient s age Vess from kidney biopsies of patients with unrelated diseases and absence of hypertension.

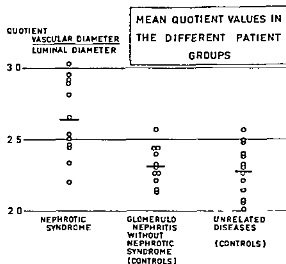


Fig 4 Mean quotient values of the patients in the different groups.

DISCUSSION

The *method of measuring blood vessel thickness* from histological sections is of course open to criticism The relatively sparse distribution of perpendicularly cut small arteries and arterioles necessitated the observation of several sections of the same sample The risk of measuring the same vessel twice seems to be equal in the different patient groups The fact that the tissue samples were of approximately the same size in all cases and that the process of fixing and embedding was standardized allows a comparison between the different patient groups



Fig 5 Normal renal arteriole Silver stain, × 450



Fig 6 Arteriole with hyperplastic and numerous cells in the wall Kidney biopsy of a patient with nephrotic syndrome Silver stain, × 450

From the results presented it becomes obvious that among the patients with glomerulonephritis there was a difference in the thickness of the small arteries and arterioles between those with and those without the nephrotic syndrome The increased cellular hyperplasia of arteries and arterioles in the nephrotic group therefore seems to be somehow related to the nephrotic syndrome

In the present series only three patients treated with corticosteroids were included The vascular changes present in the nephrotic patients of this

Table II *Coronary mortality and production of tobacco*

	Mortality from myocardial infarctions per 100 000 inhabitants						Production of tobacco per 1000 inhabitants		
	♂			♀					
	35–44	45–54	55–64	35–44	45–54	55–64	Cigars (mill)	Cigarettes (mill)	Other tobacco products (kg)
Australia	66 6	312 1	874 9	15 5	75 7	304 0	0 0049	1 69	540
Austria	36 2	162 3	513 8	10 7	36 6	155 5	0 014	1 78	120
Belgium	37 4	141 0	407 2	8 5	33 1	115 3	0 029	1 44	860
Canada	70 3	304 1	814 5	12 0	58 7	272 3	0 018	2 07	580
Denmark	35 5	177 0	547 9	5 5	30 4	163 0	0 065	1 35	670
England	57 4	222 6	684 3	8 6	41 0	198 6	0 013	2 17	300
Finland	88 6	343 9	910 5	14 3	55 3	246 1	0 0038	1 27	170
France	19 4	72 6	200 8	3 5	13 1	60 8	0 0058	1 08	400
Greece	13 1	48 9	138 9	6 7	17 3	51 3	—	1 54	—
Israel	30 8	155 2	563 8	6 2	58 9	317 9	0 00081	1 15	19
Italy	33 2	128 8	387 4	10 8	40 4	165 2	0 072	1 08	97
Japan	18 5	52 0	151 8	16 7	35 1	95 9	0 00002	1 53	16
Netherlands	33 4	164 1	495 7	4 8	23 4	129 5	0 14	1 22	1020
New Zealand	54 8	273 3	771 6	11 8	56 1	268 0	—	1 32	850
Norway	38 4	169 5	526 1	4 4	24 3	149 1	0 0036	0 38	1220
Sweden	19 9	127 2	494 4	4 4	24 8	162 1	0 0017	0 90	500
Switzerland	33 8	130 2	445 5	8 3	35 9	167 3	0 18	2 05	320
USA	90 6	358 8	923 7	18 5	81 4	313 1	0 036	2 78	400
W Germany	41 5	170 0	500 2	15 2	47 9	173 2	0 072	1 06	130
Yugoslavia	7 5	72 0	3 4 5	5 7	49 1	249 7	—	1 19	6

information was available on the consumption of various tobacco products the production figures were used as the point of comparison (Table II)

The results are shown in Table III The only significant correlation established was between cigarette production and the male and female coronary mortality rates

It is interesting that although the amount of coffee tea cocoa and alcohol consumed may be regarded at least to some extent as parameters of the standard of living no significant correlation was discernible between their consumption and the coronary mortality rate On the other hand there was a highly significant correlation between cigarette production and the coronary mortality rate but no such correlation was demonstrable for other tobacco products This correlation was equally significant with regard to both male and female coronary mortality which is different from the standard of living parameters analysed earlier

Table III *Correlation coefficients between coronary mortality rate and coffee cocoa tea and alcohol consumption and tobacco production*

	Coffee	Cocoa	Tea	Alcohol	Cigars	Cigarettes	Other tobacco products
♂							
35–44	0 090	0 19	0 32	−0 11	−0 12	0 61	0 11
45–54	0 14	−0 18	0 35	−0 17	−0 13	0 55	0 19
55–64	0 17	−0 086	0 40	−0 23	−0 11	0 52	0 18
♀							
35–44	−0 21	−0 13	0 15	−0 092	0 15	0 54	−0 34
45–54	−0 098	−0 074	0 31	−0 23	−0 18	0 61 *	−0 6
55–64	0 078	−0 060	0 38	−0 32	−0 18	0 49	−0 16

* $p<0\ 05$ $p<0\ 01$ * $p<0\ 005$

for which the significant correlation was only with male mortality Cigarette production is also correlated with the standard of living the coefficient of correlation between e g the national income and cigarette production was 0 60 ($p <$ 0 005)

It must be remembered in evaluating the results that the figures used are based on official statistics the reliability of which is not always very high Especially the extent to which the official coronary mortality rate illustrates the prevalence of coronary heart disease can and has been criticised It is clear in addition that the correlations established do not in themselves imply a causal relationship Even with these reservations I think the correlations presented here and earlier are suggestive

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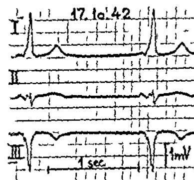


Fig 1 Case 24 Typical WPW

As to functional capacity the patients who were still alive in 1967 were grouped according to the criteria of the New York Heart Association

The death certificates of the patients who had died were requested Case records of patients who had died in a hospital were studied with special reference to cardiac state and autopsy findings The patients who had died *outside hospital had all been hospitalized within a few* years of death and these records were also obtained for study All patients seem to have known something about their cardiac disorder as in all case records great atten ion had been paid to the patient s cardiac state

The expected number of patients alive in 1967 was cal lated from tables of mortality rates in Denmark for ive year periods for each age and sex

RESULTS

I The Material as a Whole

All 47 patients could be traced In 1967 28 patients were still alive whereas 19 had died Table I shows the age and s x distribution of the patients at the age of the first ECG showin WPW and at the time of re-examination or death It will be appreciated that about half of the

Table I *Age and sex distribution of 47 patients with WPW syndrome*

Age (y)	Number of patients		
	At first ECG	On re-exam	At death
0– 9	1	0	0
10–19	2	0	0
20–29	23	0	2
30–39	6	0	2
40–49	6	8	2
50–59	4	13	3
60–69	2	3	2
70–79	3	4	5
80–89	0	0	3
Total	47	28	19
Sex	♂ 24 ♀ 23	♂ 12 ♀ 16	♂ 12 ♀ 7

Table II *Duration of PR QRS and delta waves in 46 patients with WPW syndrome*

PR (se)	No of pats	QRS (sec)	No of pats	Delta (sec)	No of pats
		0 10	3		
		0 11	4		
0 06	4	0 12	12		
0 07	3	0 13	7	0 03	3
0 08	17	0 14	11	0 04	14
0 09	7	0 15	5	0 05	5
0 10	9	0 16	2	0 06	16
0 11	3	0 17	1	0 07	7
0 12	4	0 18	1	0 08	2

patients were between 20 and 29 years of age when the first ECG showing WPW was recorded The oldest pa tient was 75 whereas in one patient (no 28) this pattern was present as early as 1924 when as a 7 year-old boy he was hospitalized because of rheumatic fever

a *Electrocardiographic findings*

The diagnosis of WPW syndrome could be confirm d in all 47 cases Table II summarizes the electrocardiographic characteristics relevant to this diagnosis It will be appre ciated that ten patients were borderline cases in respect of one of the electrocardiographic features considered charac eristic of WPW Thus P R was as much as 0 12 sec in four patients All these patients had QRS com plexes of 0 13 or 0 14 sec and delta waves of 0.04 sec or more In three patients QRS was 0 10 sec whereas P R values for the same patients were 0 08 0 09 and 0 11 sec and con picuous delta waves were present being 0 04 and 0 05 sec In three patients the delta waves were only 0 03 sec but P R interva s were 0 08 or 0 09 sec and QRS complexes 0 12 sec Further contribution to the diagnosis was offered by the fact that in all ten patients an ECG recorded on another occasion showed definitely longer P R intervals narrower QRS complexes and absence of delta waves

WPW was a concomitant finding in 20 patients who were investigated for non-cardiac diseases The remaining 27 patients were referred because of cardiac symptoms

One patient had only one ECG taken In 35 patients five ECGs or more were available and in 16 cases 10 ECGs or more were recorded In 25 of the 46 patients who had more than one ECG taken the WPW pattern was intermittent (Fig 2) and in six patients alternation between WPW and normal complexes was seen in the same ECG (Fig 3)

b *Paroxysmal tachycardia*

From the case records of the patients who had died it appeared that 11 had given a history of paroxysmal tachy cardia which was also reported by 18 of the patients still alive Thus 29 patients (62%) probably had paroxysmal tachycardia This was confirmed by an ECG in 11 pa tients Ten of them had regular supraventricular tachy cardias (Fig 4) and one had atrial fibrillation (Fig 5)

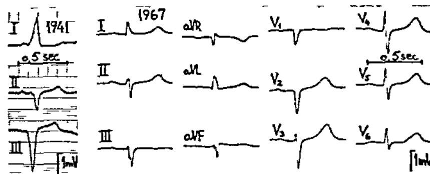


Fig 2 Case 12. 1941 typical WPW 1967 normal conduction.

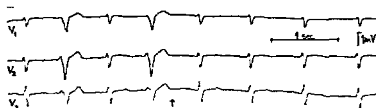


Fig 3 Case 4 Alternating normal complexes and WPW Normalization during breath holding (at arrow)

One patient had frequent extrasystoles and attacks of supraventricular tachycardia (Figs. 6 and 7)

Twenty-one patients had had attacks for more than one year and six patients for less than one year when the dia nosis of WPW was made On patient had his first attack ten years later at the age of ‚9 In one patient it was not possible to find out exactly when the attacks had started and in another information about the duration of the attacks could not be obtained. Table III shows the a͜e distribution at the first attack of tachycardia, and Tab e IV the maximal duration of the attacks. It will be appreciated that 19 patients had experienced paroxysms of one hour s duration or more although usually the duration of the attacks was 10 min or less in 20 patients, and 15–30 min in three patients.

In eight patients the attacks were invariably followed by polyuria. Three patients had experienced one or two syncopes related to tachycardia.

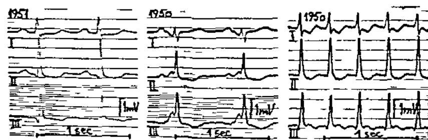


Fig 4 Case 33 1951 normal conduction 1950 WPW (center) and supraventricular tachycardia (right)

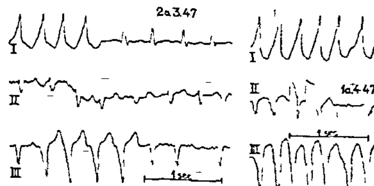


Fig 5 Case 11 Atrial fibrillation with runs of beats at a high rate and with the configurational characteristics of WPW complexes recorded in the same patient during sinus rhythm

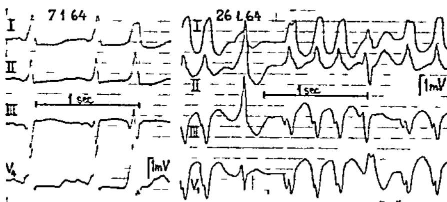


Fig 6 Case 35 WPW with single and serial extrasystoles.

As could be expected the frequency of attacks varied considerably from one patient to another and also in the same patient from time to time In seven patients it could be stated that the frequency of attacks had decreased with advancing years, whereas it had increased in eight patients Of two patients who are still alive one has had no attacks whatsoever since 1944 and another had his latest paroxysm in 1960

II Findings on Re examination of Patients Still Alive

1967 8 patients aged 44–77 years were still alive (Table I) Twenty six patients were re-examined and two answered a questionnaire The observation period was 40 years for one patient (no 28) and varied between 23 and 34 years for the remaining 27 patients

a *Electrocardiographic findings*

The ECG showed WPW in 12 patients In one (Fig 3) WPW and normal complexes alternated in a bigeminal fashion, and WPW complexes normalized during Valsalva manoeuvre or breath holding. In another patient the P waves were abnormal, being negative in leads II III and aVF pointing to retrograde atrial activation (Fig 8) QRS complexes were normal, P R intervals were 0 12 sec in leads III and aVF On most other occasions her ECG had shown WPW (Fig. 9) In still another a man aged 77 years coupled extrasystoles were periodically present (Fig 10) This finding had been reported in his case sheets as early as 1943

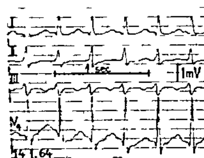


Fig 7 Case 35 Supraventricular tachycardia.

b *Paroxysmal tachycardia*

Eighteen patients had paroxysmal tachycardia In 15 patients the usual duration of the attacks was 10 min or less In three patients the attacks usually lasted 2 2–4 and 1?–24 hours respectively The maximal duration of the attacks was less than ¹/₂-hour in one patient and ¹/₂–1 hour in five patients Three patients had had occasional attacks of 24 hours duration or more The frequency of paroxysms varied considerably some patients having several attacks a week and others only one or two attacks a year

c *Functional capacity*

Eighteen patients reported no restriction of their functional capacity Nine patients aged 48–73 years belonged to functional group II their main symptoms being dyspnea on exertion and palpitation Seven of them gave a history of paroxysmal tachycardia. One patient (no 4) received an invalidity pension because of these attacks In two patients there were other than cardiac reasons why their functional capacity was restricted One a man aged 57 had had pulmonary tuberculosis for which a thoracoplastic operation had been performed. He had never had paroxysmal tachycardia Another a woman aged 50 (no 16) had chronic bronchitis arterial hypertension and slight right and left heart failure and had frequent attacks of tachycardia One patient was incapacitated by a paralysis of her calf muscles caused by a long standing lumbar nerve root compression

d *Associated findings*

One patient, a man aged 62 (no 1) gave a history consistent with earlier myocardial infarction and reported

Table III *Age at first paroxysm of tachycardia*

Age (y)	No of pats
< 0	11
20–29	1
30–39	4
45	1

Table IV *Maximal duration of tachycardia*

Duration (h)	No of pats
$<\frac{1}{2}$	4
$\geq\frac{1}{2}<1$	5
$>1<24$	13
≥ 24	5

anginal pain at rest as well as on exertion His ECG at rest in the absence of WPW did not show ischemic changes. Another man aged 49 years (no 47) had had angina on exertion for two years His ECG at rest showed WPW but no definite signs of ischemia. The above mentioned woman aged 50 (no 16) had typical angina on exertion Again the ECG at rest showed WPW but no unequivocal signs of myocardial ischemia As mentioned she also had arterial hypertension

Arterial hypertension was found in two other women one aged 51 (no 6) the other aged 66 (no 13) Patient no 6 probably had more widespread affection of the arterial system as she had rough murmurs over the carotid arteries and the aorta and inequality of carotid brachial and radial pulses.

Cardiac murmurs were heard in five patients The above mentioned woman (no 6) had a rough grade 2 systolic murmur at the apex and over the aorta. A 47 year-old man (no 28) who had had rheumatic fever had an accentuated first sound at the apex and a grade 2 mesodiastolic rumble with presystolic accentuation findings consistent with a mitral stenosis Three other patients had grade 1–2 systolic murmurs in the second and third left intercostal space

Chest roentgenograms displayed normal cardiac silhouettes in most of the patients. Only one had a slightly enlarged heart (no 4) the volume being 560 ml/m

e *Cardiac medication*

Only three patients received constant cardiac medication All three received digitalis because of paroxysmal tachycardia (nos 4 6 16) A thiazide diuretic was given to two patients with arterial hypertension (nos. 6 and 16) and nitroglycerin to one patient because of anginal pain (no 16)

III Data Concerning the Patients Who Had Died

Nineteen patients 12 men and seven women had died at ages ranging between 28 and 86 years (Table I) The observation period was 1–37 years, being 10 years or more in 12 patients Twelve patients died in a hospital and in eight of these cases an autopsy was performed

On calculating the expected mortality from the year

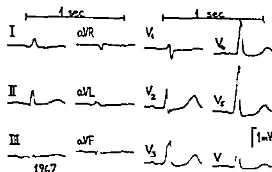


Fig 8 Case 6 Retrograde atrial activation (negative P waves in II III and aVF) in the absence of WPW

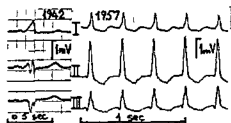


Fig 9 Case 26 1942 WPW 1957 supraventricular tachycardia

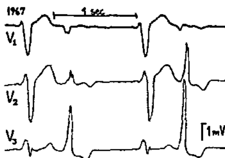


Fig 10 Case 34 WPW (1st and 3rd beats) with coupled extrasystoles (2nd and 4th beats)

of diagnosis of WPW to 1967 in each patient two patients (nos. 37 and 40) were excluded from this part of the analysis One was born in 1863 the other in 1864 and the available tables of mortality rates do not give figures for people of such old age In fact, they had died at the ages of 85 and 80 years (Table VI) With these exclusions the expected mortality for the remaining 45 patients was 10 0 In contrast to this the observed number of deaths was 17 This difference was significant ($P < 0.05$ X^2) and seemed to require further analysis

Thirty-eight patients were less than 50 years old when the diagnosis of WPW was made The expected mortality for these patients was 4 6 whereas 11 patients had actually died ($P < 0.005$ X^2) For patients more than 50 years of age at the time of diagnosis the calculated mortality was 5 5 and actually six patients had died ($P > 0.5$ X^2) (Table V)

Returning to the whole material of 47 patients 13 were 55 years or more when they died the oldest being 86

Table V *Observed and calculated mortality in 45 patients with WPW*

	Pats < 50 y at diagnosis	Pats > 50 y at diagnosis
No of patients	38	7
Observed deaths	11	6
Calculated deaths	4 6	5 5
Difference	6 4	0 5

Table VI *Thirteen patients with WPW who had died at ages between 55 and 86 years*

Case no	Sex	Age (y)	PT	HF	AH	Cause of death	Autopsy	Autopsy findings
9	♀	57	+	+	–	Cerebral thrombosis	–	
20	♂	59	–	–	–	Pulmonary embolism	+	Central pulmonary emboli thrombophlebitis of left femoral vein Slight atheromatosis of coronary arteries
21	♀	57	+	–	+	Pulmonary embolism	+	Multiple pulmonary emboli Slight myocardial fibrosis atheromatosis of coronary arteries
27	♀	86	–	+	+	Myocardial infarction	–	
30	♀	77	–	+	–	Bronchopneumonia	+	Bronchopneumonia Moderate cardiac hypertrophy moderate atherosclerosis of coronary arteries
31	♂	79	+	–	–	Cancer recti with metastases	+	Rectal cancer pleural pulmonary and suprarenal metastases Bronchopneumonia Moderate cardiac hypertrophy atherosclerosis of coronary arteries
32	♀	70	+	+	–	Pulmonary embolism	+	Central pulmonary emboli left femoral vein thrombosis Slight cardiac hypertrophy Stenosis of right coronary artery
33	♂	60	+	–	+	Cancer coli postoperative state	–	
35	♀	73	+	–		Metastatic colon cancer postoperative state	+	Cancer coli with splenic and pancreatic metastases Acute pancreatitis Normal heart
36	♀	70	–	+	–	Myocardial infarction	–	
37	♂	85	–	+	–	Paresis cordis"	–	
0	♂	80	+	uk	–	Paresis cordis	–	
	♂	55	+	+	+	Cerebrovascular insult bronchopneumonia	–	

PT paroxysmal tachycardia HF → heart failure AH = arterial hypertension uk = unknown

years Table VI summarizes some of the characteristics of these patients as well as causes of death and autopsy findings Paroxysmal tachycardia did not directly cause death in any of these cases but in two patients (nos 9 and 33) tachycardia was present terminally

Six patients died fairly young, the ages at death being 28 29 31 35 44 and 44 years Of these six patients one man aged 44 committed suicide another died at the age of 35 as a result of an electric current accident Both of these statements have been verified by forensic medical reports providing exact and unquestionable information of causes of death in both cases Neither of these two patients had had paroxysmal tachycardia or other cardiac symptoms The case histories of the remaining four patients who died young require further consideration and are given below

CASE REPORTS

Case 11

Male born 1903 This patient had no history of rheumatic fever or diphtheria He was well till 1938 when he started to have attacks of palpitation associated with precordial pain A slight enlargement of the heart was shown by physical examination and chest roentgenogram. The ECG showed WPW His condition remained unchanged till 1943 when he was taken to a German concentration camp where he was kept till 1945 Here he had frequent attacks of palpitation associated with precordial pain and dyspnea. Besides he was increasingly incapacitated by dyspnea on exertion He had long periods of diarrhea and for some time also edema of the legs In May 1945 after his discharge from the camp he was admitted to hospital because of B avitaminosis. A faint systolic murmur was heard over the apex but unfortunately no ECG or chest X ray were taken In October 1945 he had an attack of atrial fibrillation After that he remained in fairly good shape till February 1947 when he again was distressed by exertional dyspnea and precordial pain On admission to hospital in March physical examination showed a slightly cyanotic patient at rest neck vein stasis and an enlarged liver but no edema Moist rales were heard over both lungs. The arterial blood pressure was normal and no cardiac murmurs were described The heart rate was irregular at about *100/min* with a pulse deficit of 46/min The ECG showed atrial fibrillation (Fig 11) with occasional runs of beats with the configurational characteristics of WPW and similar to the ventricular complexes of earlier ECGs during sinus rhythm (Fig 5) The chest X ray showed pulmonary congestion and a considerably enlarged heart, the cardiothoracic ratio being 17/29 He was digitalized without any effect He died suddenly on April 10 1947 An ECG taken some time on that day showed atrial fibrillation with a ventricular rate going up to 240 min (Fig. 5)

Autopsy revealed a diffusely dilated and hypertrophied heart. Coronary arteries and valves were normal Micro-

scopic examination showed a pronounced diffuse myocardial fibrosis, especially subendocardially and also more extensive patches of fibrous tissue The muscle cells showed degenerative changes with vacuolization. The diagnosis of chronic myocarditis was made

Comment

This case does not seem to be one of uncomplicated WPW syndrome Unfortunately the exact frequency and duration of the attacks of palpitation or tachycardia are not stated in the records but long lasting attacks seem to have been rare as the patient more often complained of short lasting palpitation During his final illness he had a poorly controlled atrial fibrillation A slight enlargement of the heart was noted already in 1938 and this became more pronounced during the following years It is difficult to assess the role of the B avitaminosis in this setting but it may have been of some importance With proper therapy however signs of beri beri heart affection should disappear (5) Arterial hypertension was not present and coronary artery disease and valvular lesions could be ruled out on autopsy Microscopy showed a picture which is consistent with a myocardiopathy of some kind The etiology remains obscure The family history is not known

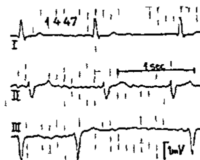


Fig 11 Case 11 Atrial fibrillation.

Case 22

Male born 1915 There was no history of rheumatic fever or diphtheria The patient was well till 1933 when after exertion he felt dizzy and vomited His pulse rate was 27/min He recovered quickly and was completely well and fit till 1943 when he started to feel extremely tired and got dyspnea on exertion He fainted 3–4 times and was taken to hospital. A faint systolic murmur was heard over the apex the pulse rate was 50/min, and a chest X ray showed an enlarged heart, the enlargement involving mainly the left ventricle Most of the 12 ECGs taken showed a short P R, broad QRS and slurring of the upstroke of the ventricular complexes (Fig. 12 (Oct. 9 1943)) Two ECGs, however showed atrioventricular block associated with another type of QRS complexes which were

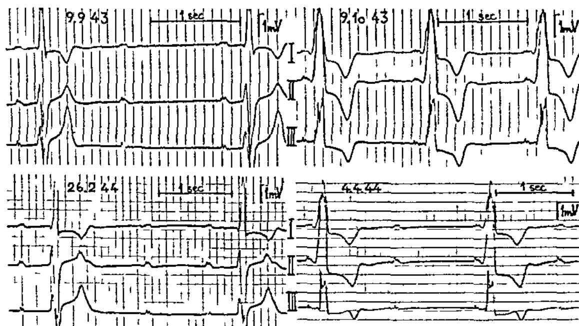


Fig 12 Case 22 Sept. 9 1943 2 1 a v block without WPW Oct. 9 1943 WPW Feb 26 1944 3 1 a v block with normal ventricular complexes April 4 1944 2 1 a v block and WPW complexes

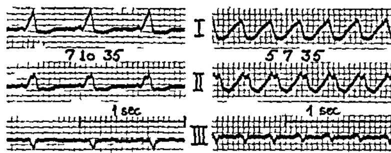


Fig 13 Case 38 Oct 7 1935 WPW July 5 1935 Regular tachycardia with abnormal intraventricular conduction

narrower On one occasion there was only a prolonged P R (0 30 sec) on another (Fig 12 (Sept 9 1943)) there was 2 1 atrioventricular conduction He was discharged without treatment

Three months later (Feb 1944) he was admitted to another hospital because of 4–5 syncopes occurring on the same day after exertion (3 football matches) His pulse rate on admission was 60/min His heart was enlarged but he was not in failure He continued to have attacks of very pronounced bradycardia and eventually died on April 10 1944 His ECGs during this admission showed 2 1 and total atrioventricular block 2 1 block was associated with both previous types of QRS complexes (Fig 12 (Feb 26 and April 4 1944)) The autopsy showed ronounced biventricular hypertrophy whereas coronary rteries and valves were normal

The family history revealed that his sister and his father had died in a similar clinical setting at the ages of 32 and 35 years respectively and the 1 year-old daughter of his brother had fainting fits

Comment

This patient did not have attacks of tachycardia The most characteristic clinical features were those of Adams Stokes attacks due to atrioventricular block enlargement of the heart and a family history suggesting that three other members of the family might have had the same disease These features strongly suggest the diagnosis of familial cardiomegaly as described by Evans (4) Association of this disease with WPW has been reported in a few cases (8)

In this case the diagnosis of WPW was made on the basis of short P R (0 12 sec) associated with broad QRS complexes (0 18 sec in lead II) and a slurred upstroke of QRS of 0 04–0 06 sec duration in the presence of normal sinus rhythm When atrioventricular block was present it was associated with narrower QRS complexes and disappearance of the delta waves During the final course of the disease however the WPW type QRS was also associated with 2 1 atrioventricular block but the P R interval of the conducted beats was invariably short (0 12 sec) as opposed to the P R interval of 0 24–0 30 sec associated with the narrower type of QRS

Case 24

Male born 1921 This patient was completely well until 194? when he experienced his first attack of tachycardia for which he was admitted to hospital On his arrival the tachycardia, which had lasted for 16 hours had disappeared The ECG showed typical WPW (Fig 1) Physical examination and chest X ray were normal During the following years he had one attack every one or two years, but from 1947 he had one or two attacks a year In 1943 1945 1947 and 1951 he was readmitted for control to Medical Department B From 1947 to February 1951 his heart volume as evaluated from his chest X ray increased from 337 ml/m to 477 ml/m² Twenty five ECGs all demonstrated WPW In 1947 he had two attacks of tachycardia in his home each lasting for 24 hours In 1948 1949 and 1950 he was admitted to a local hospital because of supraventricular tachycardia at the rate of 230/min. During all these years he remained free from other symptoms and was able to work as an agricultural labourer

In August 1951 he died suddenly in his home during an attack of tachycardia which had lasted for eight hours No autopsy was performed

Comment

At the age of 31 this patient died suddenly and unexpectedly during an attack of tachycardia He had had paroxysmal supraventricular tachycardia for 9 years often associated with a high ventricular rate The maximal frequency of attacks was two per year Usually the attacks were long lasting the maximum duration being 24 hours His heart volume had been increasing somewhat but was only 477 ml/m six months before he died All other cardiac findings were normal and he was not in failure The exact cause of death remains obscure

Case 38

Female born 1909 This patient was well till 1933 when during exertion she got an attack of tachycardia lasting

for one day During the following two years she had 2–3 attacks of rapid heart action associated with dyspnea and precordial pain. In July 1935 she had another attack which had lasted for five days when she was admitted to hospital On admission she was severely distressed with cyanosis and orthopnea neck vein stasis and liver en *largement, but no edema* Moist rales were heard over both lungs. No cardiac murmurs were described The arterial blood pressure was 95/60 The ECG showed a regular tachycardia at 230/min with broad QRS com plexes (Fig 13) The chest roentgenogram showed con siderable cardiac enlargement, the cardiothoracic ratio be ing 16/24 5 She was running a slight fever for two weeks. She was treated with digitalis and quinidine and sinus rhythm occurred on July 14 1935 The ECG then showed WPW (Fig. 13) In November 1935 she had a quite similar episode and was readmitted The course and clinical find ings were exactly the same

In January 1937 she delivered a child without any car diac complications and she remained well till October 1937 when after two weeks of tachycardia and fever she was readmitted. Physical examination disclosed neck vein *stasis liver enlargement ascites, and edema.* Pulmonary crepitation was present and the heart was enlarged The heart rate was about 200/min. During the following weeks she deteriorated gradually Her heart failure and tachy cardia were not influenced by digitalis, quinidine and diuretics Gallop rhythm and changing systolic murmurs were heard The temperature was constantly elevated and a few days before death petecchiae and ecchymoses de veloped along with bullae and furuncles. She died in December 1937 An autopsy was not performed

Comment

This patient had extremely severe attacks of tachy cardia They were long lasting and associated with a high ventricular rate of 200–230/min and pro*nounced right and left heart failure During* her final illness fever persistent tachycardia increas ing heart failure changing murmurs and pe tecchial skin bleedings were present and may sug gest the diagnosis of bacterial endocarditis al though this was not proved by blood culture Whether this was a secondary phenomenon in a severely ill patient with a low resistance remains unknown The question whether this patient had a myocardial or valvular disease could not be settled either The chest X rays all demonstrated an enlarged heart but they were all taken during long lasting attacks of tachycardia

DISCUSSION

The diagnostic criteria used in this paper cor respond to those given by other authors (1 6 13) Borderline cases are found *in all series In fact* Lamb states that specific interval figures for QRS duration and PR interval cannot be given Except in the classical form pre-excitation is best de tected by comparing the abnormal record with a normal one" (7)

It has been repeatedly stressed that, as a rule P *waves are normal in patients with* WPW On re-examination one patient in this series (no 26) had negative P waves in leads II III and aVF in the presence of normal QRS complexes As a pos sible explanation of such findings which are oc casionally encountered in these patients Scherf and Cohen suggest that occasional reversed con duction to the atria during pre-excitation may induce a transient shift of the pacemaker to the a v node (13)

The occurrence of atrioventricular block in pa tients with WPW is rare The literature on this particular subject has been reviewed by Scherf *and Cohen (13) It is stated that in most cases the* WPW pattern disappears when atrioventricular block is present However in one patient (no 22) in this series 2 1 atrioventricular block was asso ciated with persistence of the WPW pattern in the conducted beats during his final illness A few cases of this kind have been reported. In most of them the atrioventricular block occurred during an acute inferior wall infarction

Paroxysmal tachycardia was experienced by 62% of the patients in this series This is in keep ing with the figures of 40–80% given in other hospital materials and in contrast to the low fre quency of 13% found by Berkman and Lamb among 128 U S Air Force personnel (11)

Several authors state that the age at first attack of tachycardia is rarely more than 30 years (11) In this series five patients experienced their first attack at an older age (Table III)

The prognosis in the Wolff Parkinson White syndrome is generally thought to be good in the absence of associated heart disease and paroxysmal *tachycardia (1 11 13 18) On the other hand* many patients with WPW and paroxysmal tachy cardia are able to live normal active lives and longevity is experienced by a number of them However some patients are severely incapacitated by their attacks which when long lasting and frequent, may lead to heart failure In most series there are instances of sudden unexpected death among patients with WPW associated with tachy *cardia (12 13) The reason* why such patients die suddenly remains obscure As an explanation

Scherf and Cohen suggest that an impulse con ducted reversedly to the atria over one a v path way may return to the ventricles over another pathway and cause ventricular fibrillation if it arrives during the supernormal phase (13) In in fants with WPW tachycardia is often life threat ening as ventricular rates tend to be very high and association with congenital cardiac anomalies is fairly often seen (15)

American insurance companies consider the life table mortality rate to be increased by 25–30% for p ople below the age of 35 with WPW without paroxysmal tachycardia and by about 100% for people over the age of 35 In the presence of paroxysmal tachycardia the mortality rate is con sidered increased by 60–300% depending on the number duration and character of the attacks (14) In Sweden neither frequent nor prolonged attacks of tachycardia cause a premium increase for a subject with WPW (12) In Denmark the premium for persons with WPW whether associ ated with tachycardia or not equals that for people who are five years older provided that no other signs of heart disease are present (10)

In this series the observed mortality was signif icantly higher than the expected mortality (Table V) This difference was almost exclusively caused by deaths occurring among the younger patients Six deaths occurred in patients aged 44 years or less and 13 deaths in patients aged 55 years or more whereas no patients died between the ages of 44 and 55 years In the patients who died at the age of 55 years or more all deaths were prob ably unrelated to WPW Four younger patients died a cardiac death Besides WPW one of these (no 38) might have had and two (nos 11 and 22) certainly had some other cardiac disease

In a twenty-one year follow up study of 50 patients with WPW and paroxysmal tachycardia Orinius found no over mortality (12) One patient had died suddenly during an attack of tachycardia

Berkman and Lamb have recently reported the results of a five to twenty eight year follow up study of 128 U S Air Force p rsonnel (2) of whom seventeen subjects (13%) had paroxysmal tachycardia Three deaths occurred none of which —according to the authors—was ascribed to tachycardia One was self inflicted and another was due to an aircraft accident It should be ad ded that information about the third was not obtained In the absence of paroxysmal tachy cardia and signs of organic heart disease pilots who had completed their training were allowed to remain on flying status

P D White followed 100 cases and observed only one fatal case Two of the 11 patients who made up the material published in 1930 by Wolff Parkinson and White have also been followed for 27 and 38 years respectively One died at the age of 62 probably a coronary death the other is still alive and well Both had paroxysmal tachycardia and in both of them the frequency of attacks had decreased (3)

In conclusion it may be stated that in spite of the fairly high mortality figures in this small series of 47 patients the justification for the usual reser vations made regarding the prognosis in the Wolff Parkinson White syndrome should not remain un questioned More systematic follow up studies preferably including other than hospital materials are needed

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THE BEHAVIOUR OF BLOOD AND EXTRACELLULAR VOLUME IN HYPERTENSIVE PATIENTS WITH RENAL INSUFFICIENCY

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Abst act Det rminations of distribution volumes of R.I.S.A (Radio iodinated serum albumin) and bromide (as a measure of blood and extracellular volume respec tively) have been performed in 35 hypertensive patients, of whom 25 had advanced renal insufficiency

In accordance with generally accepted views the re sults were within normal limits in the ten patients with uncomplicated hypertension. In ..0 of the 25 patients w th renal failure significant elevations of either both volumes or extracellular volume alone were present

Expansions up to 5 1 of bromide di tribution volume were not evident by conventional clinical examination.

There was a good correlation between blood pressure and bromide volume expansion in patients in whom the fluid excess could be removed

It is concluded that hypertension in patients with advanced renal insufficiency may be related to fluid retention

This fluid retention is often the result of inability of the kidney to excrete salt and water and may lead to secondary heart failure

With progressive renal failure in chronic renal disease the handling of salt and water by the kidney is bound to become increasingly limited and disturbances of the balance in both direc tions may be anticipated It is well known that in the anuric patient moderate hypertension occurs in connection with over-expansion of the extra cellular fluid compartment (7 13) Observations linking water and salt retention with the produc tion of experimental hypertension in animals are abundant In normal human subjects aldosterone induced moderate salt retention is followed by blood pressure elevation (1) In patients with normal renal function or modest chronic insuf ficiency however it is generally h ld that no abnormalities of the body fluid compartments exist

In order to evaluate the state of body volume regulation in renal failure determinations were made of blood volume and bromide distribution volume in patients with advanced renal insuf ficiency and hypertension

For comparison we include our results in hypertensive patients without renal functional im pairment

METHODS

Blood volume was determined with R.I.S.A. using the volemetron apparatus The apparent distribution was corrected according to an assumed total body haema tocrit value of 0 91 times the venous haematocrit (15)

The distribution volume of bromide was determined after 1 / hours of equilibration with the same apparatus according to a method described by us previously (15) The results were found to be highly reproducible in the same individual and to correlate well with expected changes in certain pathological conditions (15) We therefore take it to be a reliable approximation of the extracellular fluid volume (6) The expected normal" values were calculated from regress on curves relating our own results in a number of normal men and women to body surface (Dubois formula)

The chromogene free creatinine in plasma was deter mined by means of the modified Jaffe reaction (8)

RESULTS

The main clinical findings of our patients are summarised in Table I Patients 1 to 10 who had hypertension not complicated by renal failure serve as a comparison Absence of cardiac failure in these patients was evidenced by the normal central venous pressure and absence of pulmonary congestion on the chest X ray As shown in Table II the absolute values as well as the ratio between blood and bromide volume do not differ significantly from normal

Table V *Blood and bromide volume increased*

Pat no		Blood volume (l)	Deviation from normal ( )	Bromide vol (l)	Deviation from normal ( )	Bromide vol / blood vol
15		5 73	+18	26 5	+26	4 6
19		4 59	+22	16 6	+12	3 6
21		6 0	+25	33 4	+45	5 6
28		5 41	+14	20 2	+2 5	3 7
29		6 30	+33	23 6	+20	3 7
30		5 80	+30	18 6	+2 3	3 2
31		6 1	+16	27 4	+21	4 5
35		6 30	+26	23 6	+19	3 7
Normal	♂	s D	8 9	s D	6	4 1
Normal		s D	12	s D	7	4 3

The second group consists of patients whose bromide volumes exceeded their calculated normal volumes by more than twice the standard deviation whereas blood volumes were normal or even lower than expected As a consequence bromide/blood volume ratios were still (with one exception) in excess of the normal value (Table V) A striking feature was the occurrence of bromide volumes up to 5 l excess without signs of oedema

In a third group we placed patients with increased blood volumes Bromide volumes were greatly increased in most of them (Table V) but in patients 28 and 30 it did not exceed the normal variation All except one (patient 19) had elevated central venous pressures Five of them (patients 15 21 30 31 and 35) also showed clinical and roentgenological signs of left sided heart failure

Some of the patients with increased bromide distribution volumes were subjected to dehydrating measures (salt restriction peritoneal or extra corporeal dialysis) until their body weight was decreased by a value approximately equal to the

Table VI *Blood pressure and volume measurements before and after dehydrating measures*

Pat no	Blood vol (l)	Difference (l)	Bromide vol (l)	Difference (l)	Body weight (kg)	Difference (kg)	Time interval (d)	Blood pressure
18	5 99	0 69	30 5	8 1	86 5	4 3	5	160–110
	5 30		22 4		82 2			130–70
20	4 71	0 21	24 2	5 4	65 1	4	9	180–1?0
	4 5		18 8		61 1			140–100
20	6 0	1 5	33 4	14 2	71 2	13 5	19	180–120
	4 5		19 2		57 7			130–100
2_	5 47	0 77	24 9	3	69 6	3 1	5	?00–120
	4 70		21 9		66 5			145–90
23	5 ?5	1 32	21 4	0 8	64 5	2 8	8	190–1 0
	3 93		_0 6		61 7			160–110
29	6 30	1 66	23 6	4 6	67 5	3	8	180–110
	4 64		19 0		64 5			140–90
31	6 1	1 0	27 4	5 9	69 5	4 8	10	170–1 0
	5 1		21 5		64 7			130–100
32	97	0 89	17 1	3 2	51 4	2 3	9	180–110
	3 86		13 9		49 1			140–95
33	5 73	1 03	26 5	7	72 2	6	20	180–90
	4 70				66 ?			140–90
34	3 88	0 27	24 6	8 1	60 3	4 8	14	210–115
	4 15		16 5		55 5			130–80

* One year after the first determination

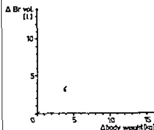


Fig 1 Relationship between change in body weight and change in bromide distribution volume in ten patients after various dehydrating measures Dashed line represents correlation that would exist if every kg weight loss were derived from 1 l bromide distribution volume

fluid excess determined. In all instances these measures resulted in a drop in blood pressure (Table VI) The fact that the feeling of wellbeing improved and no signs of dehydration appeared confirmed that the removed amount of fluid was indeed an excess The decrease in bromide volume correlated reasonably well with the observed weight loss (Fig 1)

DISCUSSION

Although there is general agreement in the literature that blood volume is normal in uncomplicated hypertension (11 12 17) there has been some discussion about the amount of extracellular fluid Grollman (10) noted a 20 % expansion of radiosulphate and mannitol distribution volumes in hypertensive patients However de Graeff (9) and Hollander (11) could not confirm these results using radiosulphate and inulin volume measurements During the past years the existence of a normal extracellular volume in uncomplicated hypertension has been generally accepted

Our results are in accordance with this current opinion Although bromide distribution volume in man is larger than those of the above mentioned substances our findings support the usefulness of bromide volume as a clinical parameter.

Our studies indicate that in far advanced renal insufficiency with hypertension expansion of either blood or extracellular volume or both are the rule rather than the exception They are in accordance with earlier reports from Schribner s group (2 3)

Boen (3) called attention to the fact that fluid subtraction in patients on intermittent haemodialysis is often followed by lowering of elevated blood pressure He stressed that the time interval before the blood pressure reacted to this treatment varied considerably in different patients This may be one of the reasons why there is no general agreement as to the efficacy of fluid subtraction in these patients

Blumberg et al (2) found elevated values for bromide and Na^{+} distribution volumes in patients with far advanced renal failure both with and without intermittent haemodialysis treatment In both groups satisfactory blood pressure control was achieved when Br^{8} and Na^{+} distribution volumes were reduced to near normal levels Merrill et al (14) had also shown a close correlation between sodium balance and blood pressure in bilaterally nephrectomised patients

In the totally anuric patient treated by periodic haemodialysis any expansion of the body fluids is evidently the results of failure of excretion by the kidney In the anephric dog blood pressure correlates with the state of hydration (16). Observations by the team of Borst (4) extensively documented that in man with intact kidneys fluid retention is followed by hypertension This was also clearly shown in the aldosterone treated subjects of August & Nelson (1) although the authors did not comment upon this point As all of our patients were in preterminal state (though urine volume in most of them was still maintained) the inability to excrete more than a minimal amount of salt and water resulting in positive fluid balance is the most likely explanation in this type of patient too

It has been noted by Hollander (11) that only hypertensive patients with congestive heart failure had an expanded sulphate volume Though heart failure may undoubtedly occur as a result of hypertension and subsequently cause fluid retention primary fluid retention by the kidney will eventually result in congestive failure and the clinical end result may be indistinguishable The fact that correction of the fluid excess causes the blood pressure to drop is suggestive of the fact that fluid retention presumably of renal origin was the primary occurrence

In Hollander s study (11) six of the ten patients with malignant hypertension showed expanded sulphate volumes In only one of them was venous pressure elevated but they all had

prolonged circulation times These patients had little renal functional impairment Blood volumes were not measured but were probably increased having regard to the circulation times The authors considered them to have no impairment of sodium excretory capacity

In any case our patients shown in Table IV belonged to a clinically different group (they all had severe renal failure) They did not have increased blood volumes which in itself makes heart failure improbable The 20 patients who showed abnormal volume determinations undoubtedly constitute a heterogeneous group and have been arbitrarily divided according to their blood volumes into the groups represented in Tables IV and V All of these uraemic patients had anaemia the haematocrit values ranging from 19 to 31% in patients 17–35 Only patients 15 and 16 had haematocrit values of 41 and 39% respectively The values for total serum protein and albumin content varied among the different patients being usually in the lower range without reaching nephrotic" values Patient 27 whose values for blood and bromide volume contrasted sharply (Table IV) showed the lowest albumin concentration (22 g per l) which certainly contributed to this extracellular fluid excess In the other patients no clear correlation existed between the serum albumin level the haematocrit value the nature and the degree of renal insufficiency and the patterns of volume abnormalities We believe that these and other partly unknown factors (like previous diet) although contributing to the disturbance were too manifold to permit their respective evaluation in a small number of patients

Some of the patients in Table IV probably represent a later stage than those in Table III whereas in those with relatively expanded blood volumes (low bromide volume/blood volume ratio) cardiac failure certainly predominated and may have contributed to the fluid retention In favour of this assumption is that in the patients whose bromide volumes were within normal limits attempts to lower blood pressure by further fluid subtraction resulted in severe orthostatic complaints impairment of wellbeing and sometimes a rise of blood urea levels The finding of Shibagaki (18) that kidneys of patients with terminal renal failure may have elevated renin contents suggests that abnormal release of renin by the diseased kidney may be related to hypertension even in patients with far advanced disease

In many patients however as shown in Table VI correction of fluid excess improved the blood pressure and the general condition It is important to realise that significant but clinically unsuspected fluid retention may be present and is often related to blood pressure elevation In view of the grave prognosis of most patients in this group treatment of this aspect alone is not always very rewarding but from our experience it may result in remarkable subjective and objective improvement

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HEREDITARY COPROPORPHYRIA

A Family with Unusually Few and Mild Symptoms

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Abstract A family with hereditary coproporphyria from central Jutland Denmark is described In spite of excretion of considerable amounts of coproporphyrin III with the feces but no protoporphyrin and occasionally increased urinary porphyrin as well as porphyrin precursors (PBG and/or ALA) in the urine all the affected members except one were completely free from symptoms of porphyria during the four years the family was under observation The only member of the family exhibiting symptoms had a mild attack of acute intermittent porphyria type presumably provoked by barbiturates accompanied by pyelonephritis and coli sepsis She constantly excreted high amounts of coproporphyrin III in her feces and periodically at least increased coproporphyrin and slightly increased uroporphyrin with the urine In most of her siblings the excretion was intermittent and in all of them it was considerably lower than in the patient although highly elevated Among the next generation the excretion was much lower The observations support the concept of dominant inheritance

In a recent paper Connon and Turkington (1) described a case of hereditary coproporphyria (HCP) with photosensitivity and episodes of jaundice and four latent cases her father and all her three siblings Furthermore mental disorders occurred in the family These authors emphasize that HCP in contrast to earlier reports is far from always of a benign nature We therefore find it appropriate to publish in some detail our studies through four years of an essentially benign form of HCP occurring in a family in central Jutland as this family has earlier been reported on only very briefly and insufficiently (3) Case history and family studies follow below The chemical technique employed was that described in detail by With (2) The clinical examination of the family members was performed by one of us (J C L) the chemical analyses and preparations by the other (T K W)

CASE REPORT

A S woman born Dec 8 1908 unmarried no 110 in the Danish porphyria register Admitted on June 11 1964 to the Surgical Department of the County Hospital Holstebro for appendicitis S nce March 1964 she had suffered from sciatica like pains On June 1 she began to take a composite mixture containing phenemal This was followed by diffuse abdominal pains and vomiting After transient amelioration the pains came back and she was admitted as acute patient with abdominal pain constipation and tachycardia. On June 15 it was noted that her urine was reddish and porphyrinuria was found after which she was transferred to the Medical Department on June 18 Examination then showed a blood pressure of 240/130 pul e 112 and temperature 37 6 C The following days she gradually became mentally confused and her temperature rose to 39 4 C on Jun 24 Urine examination had shown a pyelonephritis and blood culture showed growth of coli bacilli for which antibiotics were administered Her temperature began to fall on June 25 and reached normal on June 30 Her critically high blood pressure gradually fell to between 200 and 150 systolic and between 130 and 100 diastolic On July 6 antibiotic treatment was stopped which was followed by a slight increase in temperature until July 25 when a steep rise of temperature to 39 C occurred After repeated antibiotic treatment the temperature became normal within a week Because of the high initial blood pressure she was suspected of pheochromocytoma, but studies of urinary catecholamines did not support this diagno is Her ECG was normal except for left a is deviation and X ray of her thorax showed nothing abnormal Pyelography (i v and direct) disclosed a double renal pelvis on the left side but was otherwise normal She was discharged on Oct 26 relieved of all her complaints except a slight uncomplicated hypertension which was not regarded as requiring specific treatment.

Po phyr n St d es on the Pat ent (A 3 n Table I)

After porphyrin had been demonstrated in the urine of the patient her excreta were sent to the laboratory of Svendborg County Hospital where her urine was found to contain 440 μg coproporphyrin per 100 ml and 20 μg uroporphyrin per 100 ml as well as PBG 1 0 and

Table I *A = first generation B = second generation*

A 1 dead at time of investigation
A 2 died from cancer of the large intestine in 1966

Subject	Sex	Born	Offspring
(A 1)	♂	1905	
A 2		1906	
A 3	♀	1908	
A 4		1915	
A 5	♂	1915	B 5 1 ♂
			B 5 2 ♀
			B 5 3 ♂
			B 5 4 ♂
A 6	♂	1917	B 6 1 ♂
			B 6 2 ♀
			B 6 3 ♀
			B 6 4 ♂
			B 6 5 ♂
A 7	♂	1920	
A 8		1923	
A 9	♂	1926	
A 10	♀	1929	B 10 1 ♀
			B 10 2 ♂

Within brackets = no chemical studies of subject in question
Without brackets or underlining = chemical studies on urine and feces performed and found normal
—— = Latent carrier i e abnormal values in urine and or feces or carriers among the offspring
═══ = at least one attack of AIP type

ALA 0 65 mg per 100 ml, while her feces contained as much as 1800 μg coproporphyrin per g of dry substance all the porphyrin being coproporphyrin The porphyrin from her feces was prepared as purified ester and exhibited characteristic conglomerates of very short rather coarse crystal needles and the low melting point (1_0) of the crystals characteristic of coproporphyrin III ester A family study was planned the result of which is presented in Table I

Porphyrin Studies on the Family (Symbols see Table I)

In August 1964 the findings were as follows *A 2* normal porphyrin PBG and ALA in urine but in feces coproporphyrin 268 μg per g dry substance *A 3* patient, see above *A 4* normal conditions in both urine and feces *A 5* urine normal feces 340 μg coproporphyrin per g dry matter *A 6* urine showed negative screening test for porphyrins, PBG 0 15 ALA 0 60 mg 100 ml and feces coproporphyrin 340 μg per g dry substance *A 7* and *A 8* normal findings *A 9* urine normal feces 335 μg per g dry matter *A 10* urine normal, feces 80 μg per g dry matter

B 5 1–B 5 4 aged 22–12 years were studied in November 1964 *B 5.1* showed normal urine but coproporphyrin 80 μg per g dry matter in feces. *B 5.2* showed urinary PBG 0 4_ and ALA 0.95 but normal fecal porphyrin. *B 5.3* and *B 5 4* both showed slightly elevated urinary PBG (0.3 and 0.35 mg/100 ml respectively) but otherwise normal analytical data *B 10.1* and *B 10.2* aged 13 and 10 years both showed slightly elevated PBG (0 40 and 0.55 respectively) but otherwise normal findings. *B 6 1–B 6.5* aged 20 to 6 years were studied in April 1965 showed normal excretion and were clinically normal

During April 1965 *A 6* was studied again and showed coproporphyrin 880 μg per g dry matter in feces, but normal urine analysis for porphyrins PBG and ALA

During November 1965 *A 2* was studied and showed PBG 0.24 and ALA 4 0 mg/100 ml in urine the feces were not studied on this occasion

During October 1966 *A 3* showed PBG 0 63 mg/100 ml but normal ALA and coproporphyrin in feces 1765 μg per g dry matter *A 4* showed PBG 0 75 mg/100 ml on this occasion but normal ALA and fecal porphyrins *A 7* showed no findings in urine but coproporphyrin in feces 155 μg per g dry matter *A 8* exhibited normal urine and feces values *A 10* showed normal ALA but slightly elevated PBG (0 30 mg/100 ml) and fecal coproporphyrin 430 μg per g dry matter

During May 1967 *A 10* was studied again and showed normal urinary PBG negative screening test for porphyrins in urine and ALA 0 75 mg/100 ml *Fecal* coproporphyrin was 650 μg per g dry matter

During July 1968 *A 3* showed slightly elevated PBG (0 36 mg/100 ml) and normal ALA urinary coproporphyrin 70 and uroporphyrin 15 μg per 100 ml Her fecal coproporphyrin was 1400 μg per g dry matter *A 6* had normal urine but fecal coproporphyrin 245 μg per g dry matter *A 10* showed PBG 0 95 but normal ALA and negative screening test for urine and fecal porphyrins

During the summer of 1964 *one of us* (J C L.) contacted the family members *A 2 A 4 A 5 A 6* and *A 10* as well as eleven of the members of the second generation *B 5 1–B 5 4 B 6 1–B 6.5 B 10 1* and *B 10.2* None had any complaints suggesting acute porphyria attacks and no photosensitivity skin fragility or abnormal pigmentations

DISCUSSION

The essential benignity of the type of HCP prevailing in this family is evident It is noteworthy that patient *A 3* had highly elevated coproporphyrin in feces on all occasions when it was determined and that also her urinary copro- and to a lesser degree uroporphyrin was elevated on the two occasions when it was measured Her PBG was also increased on all three occasions when it was measured but ALA was only increased on the first study during her attack Some of her siblings showed increased ALA with normal PBG (*A 6* in 1964 *A 10* in 1967) while others had normal ALA with increased PBG (*A 4* in 1966 *A 10* in 1966 and 1968 as well as *A 3* herself in 1966 and 1968) In *A 4* the fecal porphyrin screening test was negative on the two occasions when she was studied (1964 and 1966) for which reason the character of this disease cannot be reliably determined on the basis of fecal analysis alone because on one occasion

(1966) she had definitely elevated urinary PBG In some of the latent cases (*A 6*) the fecal porphyrin was constantly elevated as found in patient *A 3* while in others (*A 10*) the fecal porphyrin excretion was intermittent

The excretion in members of the second generation—aged 22–6 years—was much lower than in the first generation and only in one of them was the fecal coproporphyrin elevated and only to a very moderate degree contrary to the urinary excretion of precursors which was slightly elevated in a high percentage of the few members of the second generation studied an elevation just above normal limits

It is surprising that only one (*A 8*) of the members of the first generation subjected to study showed normal conditions in all the tests employed (screening for porphyrins in urine and feces ion exchange chromatographic determination of PBG and ALA quantitative porphyrin analyses if the screening tests were positive) In the second generation six of the 11 subjects studied exhibited minor increases either of the excretion of porphyrin precursors with urine or of fecal porphyrins The same high incidence of latent cases was found by Connon and Turkington (1)—all three siblings and the father

The only porphyrin present in the feces was coproporphyrin protoporphyrin was never found in analyses including thinlayer chromatographic studies (4 6) The porphyrin was prepared as crystalline ester from the feces of *A 3* *A 5* and *A 10* and in all three the crystals were ball like conglomerates of short needles (hedgehogs) with melting point between 120 and 140 C typical of coproporphyrin III tetramethyl ester

This family study differs from most others in being continued over several years and has disclosed intermittant excretion in several of the latent carriers Although fecal porphyrin excretion is the dominant chemical symptom in this family it is not constant and sometimes the feces were normal while the urinary excretion of precursors was elevated In one (*A 4*) her status as a carrier could only be found on urinary analysis as her only abnormality was a definitely increased PBG on one occasion

The findings stress the necessity of always studying both urine and feces and of employing ion exchange analysis for the precursors as previously made clear by one of us (3 5)

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SERUM IMMUNOGLOBULINS AND ORGAN SPECIFIC CELLULAR HYPERSENSITIVITY IN ULCERATIVE COLITIS AND CROHN S DISEASE

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Ab tract The serum concentrations of IgG IgA and IgM have been measured in 2? patients with ulcerative colitis and in 23 patients with Crohn s disease. The con e ration of IgG was significantly higher in ulcerative colitis than in Crohn s dis ase but the examination d d not show any major differences between the two conditions or alterations from the normal In Crohn s dis ase the serum concentration of I G was lower in patient treated than in patients not treated with glu ocorticoid A similar difference was not found in ulcerative colit

In a paral el study on the same patients the in vitro inhibition of leucocyte migration induced by jejunoileal and colonic mucosa components wa examined A re activity which is probably an in vitro correlate to organ specific cellular hypersensiti ity to intestinal mucosa components, was demonstrated in a ma ority of the patients with ulcerative colitis, whereas pat ents with Crohn s disease d d not differ sign ficantly from contro s There was no correlation between the serum concentration of the various immunoglobulins and the result of the leucocyte migration test.

As important fluctuations in production, catabolism extravascular deposit, and loss of immunoglobul ns may be concealed beh nd comparatively normal s rum immunoglobulin concentrations, more conclusi e results may be gained from examinations which include metabolic studies of the immunoglobul ns.

The etiology of ulcerative colitis and Crohn s disease is still obscure but immuno ogical aspects have become increasingly important in pathogenetic considerations (7 8) Often a distinction between the two conditions can be drawn on the basis of conventional nosographic criteria, but if only the colon is involved the classification is difficult and may be impossible

If however a classification is made according to existing criteria ulcerative colitis seems to be associated with a hyperactive immune organ (4 5 12) whereas Crohn s disease is possibly associated with r duced immuno ogical responsive ness (1 9 12) Although th re is no general agreement on this hypothesis (6) it was found of interest to examine wh th r a difference of immunological reactivity is reflected in the absolute or relative serum concentrations of the ma or immunoglobulins IgG IgA and IgM

Further as organ specific cellular hypersensitivity to intestinal mucosa compon nts is gener ally not found in Crohn s disease but appears to be an immunopathological feature of ulcerative colitis (1) it was resolved to examine the oc currence of cell mediated auto-reactivity and its correlation to the immunoglobulin pattern by means of leucocyte migration tests in the same patients

MATERIAL

The different al diagnosis between ulcerative colitis and Crohn s disease was based upon clinical features, rad ological examination, recto-sigmoideoscopy and histopathological examination of b opsy specim ns or tissue removed by operation. Cases which could not be classi fied with certainty were excluded.

Ulcerat e col tis was diagnosed in ?2 patients ten females (age 10–66 avera e 36 years) and 1. males (a e 13–63 avera e 35 years) All cases showed signs of activity with haemorrhagic diarrhoea, fever abdominal pain, hypersedimentation, and leucocytosis In four pa tients subtotal colectomy had previously been under taken Nine patients recieved treatment w th glucocorti co ds at the time of study

C ohn s dis ase was diagnosed in 23 patients 13 females (age 16–65 average 37 years) and ten males (age 15–74 a era e 35 years) All cases were more or less active with diarrhoea, abdominal pain fever and

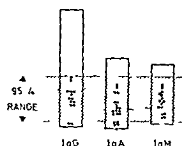


Fig 1 The serum immunoglobulin in 22 patients with ulcerative colitis compared with normal controls The absolute values are given in the text

hypersedimentation In six cases the changes were located to the terminal ileum in 11 cases only the large bowel was involved and six had a combined ileo-colic involvement. In five patients intestinal resection with ileo transversostomia had been performed Six patients received treatment with glucocorticoids at the time of study

METHODS

The concentrations of IgG IgA and IgM in sera stored at −18 for 2 months–2 years were estimated by electrophoresis in antibody containing agarose (13 14) Human Standard Serum Op no 166 (Behringwerke Marburg, Lahn Germany) was used as quantitative reference The rabbit antibodies used were antihuman IgG (Dakopatts Ltd Copenhagen) antihuman IgA and antihuman IgM (Behringwerke Marburg Lahn, Germany) The serum levels of IgG IgA and IgM were determined in 44 healthy members of the medical staff (average age 33 years) and 55 blood donors (average age 39 years) The distribution proved to be positively skew but the log. transformed values showed a normal distribution In all the calculations therefore log. values were used The 95 range was for IgG 7 2–15 1 g l (mean 10 4 g l) for IgA 0 74–3 06 g l (mean 1.50 g/l) and for IgM 0.23–1.33 g l (mean 0.56 g l)

Leucocyte migration test (LMT) Organ-specific cellular hypersensitivity was estimated by means of the leucocyte migration test (LMT) which has been described in detail elsewhere (3 11) Washed peripheral leucocytes are allowed to migrate for 24 hours from the open end of a 1 4 mm capillary tube along the plain bottom of a 1 ml tissue culture chamber The circular migration area is measured by planimetry The migration areas in a series of cultures without antigen are put in relation to the migration areas of a parallel series of antigen-containing cultures The migration index (MI) hereby calculated indicates an inhibition if the value is below and a stimulation if the value is above unity

Pooled extracts of fetal colonic or jejunoileal mucosa were used as antigens Extracts of fetal liver and kidney were used as controls to ensure organ-specificity The tissue extracts were standardized by protein determination The concentration of intestinal mucosa antigen in the cultures was as routine 50 μg protein per ml culture medium.

The MI values of 55 controls examined with intestinal mucosa extracts showed a normal distribution The 95% range with colon mucosa (mean 0 95 ± 2 S.D) was 0 79–1 11 with small intestinal mucosa (mean 0 94 ± 2 S.D) 0 78–1 10

RESULTS

Serum levels of IgG IgA and IgM

The serum levels of IgG IgA and IgM are presented in Figs 1 and 2

In ulcerative colitis the mean concentration of IgG was 12 4 g/l (95% range 5 5–27 8 g/l) of IgA 1 33 g/l (95% range 0 38–4 63 g/l) and of IgM 0 54 g/l (95% range 0 18–1 60 g/l) The mean serum concentration of IgG is significantly different from the controls ($p < 0.05$) The standard deviations of IgG ($p < 0.01$) and IgA ($p < 0.05$) are different from the normal and there is a positive correlation between the serum concentrations of IgG and IgA ($p < 0.01$)

In Crohn s disease the mean concentration of IgG was 9 7 g/l (95% range 4 4–21 5 g/l) of IgA 1 25 g/l (95% range 0 36–4 33 g/l) and of IgM 0 49 g/l (95% range 0 17–1 46 g/l) The mean values do not differ significantly from the controls ($p > 0.05$) According to variation analysis the deviations in the IgG ($p < 0.01$) and IgA concentrations ($p < 0.01$) are significantly different from the normal As in ulcerative colitis there is a positive correlation between the serum concentrations of IgG and IgA ($p < 0.05$)

A comparison between the serum concentration of IgG IgA and IgM in ulcerative colitis and Crohn s disease shows a significantly higher serum concentration of IgG in ulcerative colitis ($p < 0.05$) but no difference as far as IgA and IgM are concerned

Leucocyte migration test (LMT)

The migration indices in the two groups of patients appear from Fig 3

In accordance with previous observations (1)

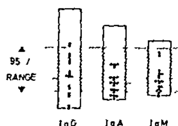


Fig The serum immunoglobulins in 23 patients with Crohn s disease compared with normal controls The absolute values are given in the text

it was found that specific inhibition of the migration was induced by jejunoileal as well as colonic extracts The lowest value observed in each patient is indicated in the figure

In ulcerative colitis the mean MI (0 70) was significantly different from the normal ($p < 0\ 001$) and from the Crohn group ($p < 0\ 001$) Six patients had migration indices within the normal range

Comparison between serum immunoglobulin levels and the leucocyte migration inhibition

In Fig 4 the migration indices and the serum immunoglobulin levels in ulcerative colitis are compared No significant correlation exists between the MI and the serum concentration of IgG IgA and IgM ($p < 0\ 05$) The group of patients under glucocorticoid treatment does not differ from the rest as regards immunoglobulin levels and migration indices ($p > 0\ 05$)

In Fig 5 the migration indices and the serum immunoglobulin levels in Crohn s disease are compared No significant correlation exists between the MI and the serum concentration of IgG IgA and IgM ($p > 0\ 05$) Patients treated with glucocorticoid had significantly lower serum concentration of IgG ($p < 0\ 01$) while IgA and IgM as well as migration indices did not differ from the remainder The patients with affection exclusively of the terminal ileum did not differ significantly from the patients with colonic or combined ileo-colic involvement as regards serum concentration of the three immunoglobulins and migration indices

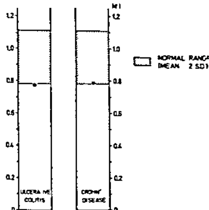


Fig 3 The leucocyte migration indices in 22 patients with ulcerative colitis and 23 patients with Crohn s disease compared with normal controls

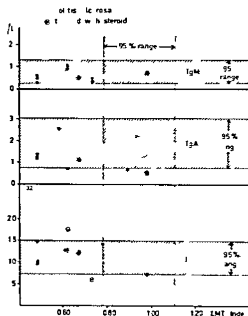


Fig 4 The relation between leucocyte migration indices (x) and the serum concentrations of IgG IgA and IgM (y) in 22 patients with ulcerative colitis The patients treated with glucocorticoid are indicated in the figure

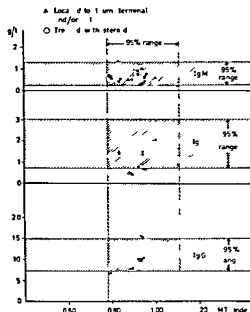


Fig 5 The relation between leucocyte migration indices (x) and the serum concentrations of IgG IgA and IgM (y) in 23 patients with Crohn s disease The location of the disease as well as treatment with glucocorticoids are indicated in the figure

DISCUSSION

Previous studies of the serum protein pattern in patients with ulcerative colitis and Crohn's disease have shown that the gamma globulin level estimated by paper electrophoresis may be above within or below the normal range (4 5 10) and a similar variation of IgG levels should be expected (14) The IgG as well as the IgA values showed a greater deviation from the normal mean than the controls and the study further showed a higher level of serum IgG in ulcerative colitis than in Crohn's disease

The serum concentration of an immunoglobulin is an effect of a dynamic balance between on the one hand production and on the other destruction loss or extravascular deposit of the protein In ulcerative colitis or Crohn's disease this balance is not influenced in a way which is reflected as typical consistent changes of the serum concentrations of IgG IgA and IgM This finding however does not exclude the possibility that profound changes in the immunoglobulin turnover may exist

The importance of parallel metabolic studies is clearly seen from examinations of IgG turnover in patients with Crohn's disease (2) which showed that a considerably increased production and degradation was concealed behind normal or slightly abnormal serum levels of IgG It cannot be excluded that similar marked changes of IgG turnover exist in ulcerative colitis and in both conditions IgA and IgM may be involved as well This however can only be decided on the basis of metabolic studies with pure preparations of these compounds Such studies are presently being undertaken and might serve to elucidate further the immunopathological difference between ulcerative colitis and Crohn's disease

The results of the LMT using extracts of fetal colonic and small intestinal mucosa confirm the existence in ulcerative colitis of an organ-specific cellular reactivity which is probably an in vitro correlate to cellular hypersensitivity to intestinal mucosa components although the reaction is not present in all cases A similar reactivity is a rare feature of Crohn's disease also in cases with colonic involvement and seems to be another example of different immunological reactivity in the two conditions The lack of correlation between the capacity for organ specific cellular reactivity and the serum concentrations of the various immunoglobulins was not unexpected but does not allow any final conclusion until further studies of the metabolic pattern of the various immunoglobulins have been undertaken

Glucocorticoid treatment did not seem to influence the serum immunoglobulins in ulcerative colitis whereas in Crohn's disease the serum concentration of IgG during glucocorticoid treatment was significantly lower than in untreated patients This finding might indicate that glucocorticoids influence the IgG metabolism differently in ulcerative colitis and Crohn's disease Organ specific cellular hypersensitivity as measured by the LMT was not significantly influenced by glucocorticoid treatment

The conclusion of the present study is that although marked alterations of the production catabolism loss or deposit of immunoglobulins should be expected in ulcerative colitis and Crohn's disease such disturbances if present do not induce any characteristic change of the absolute and relative concentrations of IgG IgA and IgM in the serum and that further information has to be gained from examinations which include metabolic studies of the individual immunoglobulins

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DISSEMINATED INFECTION CAUSED BY MYCOBACTERIUM KANSASII

Report of a Case and Brief Review of the Literature

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Abstract A case of disseminated visceral infection with Mycobacterium kansasii in a previously healthy 23 year old woman is reported The clinical picture was dominated by fever granulocytopenia and weight loss Later severe pancytopenia and splenomegaly were outstanding. The course was fatal in spite of therapy with antituberculous drugs. The association of blood dyscrasias in disseminated mycobacterial infections in man is pointed out. The litera ture is briefly reviewed

The so called atypical mycobacteria which were originally considered saprophytes or commensals nonvirulent for man have proved to be the cause of both pulmonary and extrapulmonary infection Pulmonary lesions are by far the most frequent but localized extrapulmonary infection such as suppurative cervical adenitis in children (5 29) cutaneous infection (6 17) and bone and joint involvement (5 6 15) have been reported Disse minated infection due to these organisms has been reported to have an almost invariably fatal course (3 8 14 16 18 20 23 24 27 28 30 31 32) In cases of disseminated disease morphological changes in peripheral blood and hepatosplenomegaly are frequent findings and it is often pre sumed that the patients have a primary disease in blood forming tissues The purpose of this paper is to report on a case of disseminated vis- infection with Mycobacterium kansasii (Run yon Group I photochromogenic) (22) associated with pancytopenia and hepatosplenomegaly and to review briefly the literature To our knowledge this is the first case to be reported from the Scan dinavian countries

CASE REPORT

A 23 year-old married woman was in April 1967 trans- ferred from the Hospital of Infectious Diseases to the Department of Medicine Sahlgren s Hospital Goteborg because of persistent fever anemia and granulocytopenia

Since adolescence she had inclined to have a mild ane- mia probably due to large menstrual blood losses. Partu rition 5 and 2 years before was normal. In the spring of 1966 she had a spontaneous abortion. Otherwise the past history was negative Since childhood she had been living in Goteborg and she had never been abroad. There was no familial occurrence of tuberculosis nor was the disease known to exist in her immediate environment.

At the beginning of March 1967 there was an onset of cough without expectoration, fatigue anorexia, mild jaun dice and fever to 38.5 C On March 30 she was admitted to the Hospital of Infectious Diseases where physical examination revealed a moderately icteric female who did not appear acutely ill The lungs were clear There was no lymphadenopathy the liver and spleen were not pal pable but X ray examination showed a slight increase in the size of the spleen. Chest X ray showed bilaterally small infiltrations in the hilar regions The laboratory findings were hemoglobin 10 0 g/100 ml, hematocrit 30% RBC 2 9 mill./mm reticulocytes 2.4% WBC 1400/mm and a platelet count of 190 000/mm³ A bone marrow aspiration did not yield enough material for evaluation ESR was 87 mm/h. Total bilirubin 4.5 mg/100 ml alka line phosphatase 13 Buch Buch units (normal 2-8) thymol turbidity test, SGOT and SGPT were normal. Serum hap- toglobin 247 mg/100 ml serum iron 55 μg/100 ml, and TIBC 375 μg/100 ml Direct and indirect Coombs tests were negative There were no cold agglutinins in the serum. Complement fixation tests for enterovirus and PPLO were negati e B fore institution of therapy e ght blood cultures were performed and all proved sterile In spite of treatment with antibiotics (penicillin, tetracycline s reptomycin) fever (38 -39 C) anemia and granulocyto- penia persisted but jaundice disappeared and chest X ray returned to normal

Table I *Results of test for* Toxoplasma gondii *antibody*

Date of collection		Sabin–Feldman dye test	Complement fixation test
March	31	1/500	1/32
April	10	1/500	1/32
May	2	1/250	1/64
May	3	1/250	1/16
May	23		1/32
July	19	1/250	neg

On April 21 she was transferred to the Department of Internal Medicine At examination she appeared chronically ill The liver edge was palpated 2 finger breadths beneath the right costal margin on deep inspiration Otherwise the physical findings were negative Laboratory tests revealed hemoglobin 10 1 g/100 ml hematocrit 35% RBC 4 0 mill/mm³ reticulocyte count 4 0% WBC 1900/mm with a percentage of 6 stabs, 68 segmented neutrophiles 1 eosinophile 1 monocyte and 24 lymphocytes The platelet count was 156 000/mm The urine sediment and serum creatinine were normal. The serum haptoglobin was 10 mg/100 ml and the ESR 50 mm/h Repeated tests for occult blood in the stools were negative Serum iron 60 μg 100 ml and TIBC 330 μg 100 ml The serum vitamin B_{12}, whole blood folic acid and serum folic acid levels were normal Total bilirubin and thymol turbidity tests were normal but alkaline phosphatase was substantially elevated (39 Buch-Buch units) SGOT and SGPT were mildly elevated Bromsulphthalein retention after 45 min was 16% The total serum protein was 5 7 g/100 ml with albumin 2 3 α–0 4 α_2–0 5 β–0 9 and γ-globulin 1 6 g/100 ml Serum immunoelectrophoresis was normal Direct and indirect Coombs tests were negative Repeated LE cell tests were negative Serological tests for syphilis typhoid and brucellosis were negative No cold agglutinins were found Complement fixation tests for adenovirus ornithosis and Coxsackie B viruses, and for Histoplasma capsulatum were negative Paul Bunnel s test was negative Dye tests and complement fixation tests for Toxoplasma antibodies were positive (Table I) Endometrial tissue liver tissue and blood were inoculated intraperitoneally into 6-week-old mice but Toxoplasma gondii was not isolated The Mantoux reaction (1 1000) was positive and the histoplasmin skin test negative Repeated blood cultures were sterile

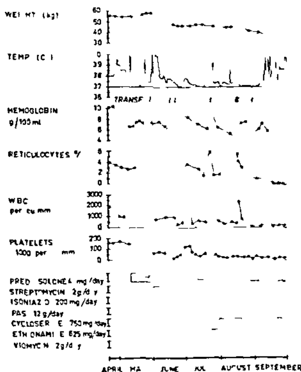


Fig 1 Course of the disease and treatment

Sternal marrow aspirations performed on April 24 June 12 and July 13 yielded sparse material and the smears showed hypocellularity probably due to the admixture of peripheral blood There was a relative increase in erythropoiesis but a marked decrease in granulopoiesis The average differential count of the bone marrow smears showed a percentage of 3 proerythroblasts 53 erythroblasts, 1 myeloblast 8 myelocytes 19 metamyelocytes and granulocytes 13 lymphocytes, 1 monocyte and 2 plasma cells Histological examination of biopsy specimens from spinal processes however revealed a hypercellular marrow practically without fat. There was a marked erythroid hyperplasia, normal number of megakaryocytes but a granulocytic hypoplasia There was a discreet increase in the amount of reticulin fibrils Granulomatous lesions were not found

She remained in the hospital until she died on September 29 The course of her illness is illustrated in Fig 1 Initially the clinical picture was dominated by fever (38 0 –39.0 C) malaise nonproductive cough, progressive anemia and granulocytopenia. The primary suggestion was that she had a chronic infectious disease or a systemic granulomatous disorder such as Hodgkin s disease or both Laboratory investigation for tuberculosis was started on May 5 (Table II) and prednisolone 40 mg orally per day was instituted on May 11 On May 27 a morbilliform nonpruritic skin eruption appeared over the whole body persisted for two weeks and then disappeared. Biopsy of the skin lesions was not performed Examination of a liver biopsy specimen taken on May 19 revealed discreet infiltration of periportal spaces with lymphoid cells and mild fatty metamorphosis. A few small granulomata of epitheloid histiocytes, some lymphocytes and polymorphonuclear leukocytes without central necrosis were found Sections stained for acid fast bacilli (Ziehl Neelsen) were negative

At the end of May she again developed jaundice with a bilirubin level of 3 8 mg 100 ml At the same time there was a fall in the hemoglobin concentration, a rise in the reticulocyte count, a low haptoglobin level and spiking fever The anemia required blood transfusions. Because of the suspicion of disseminated tuberculosis, streptomycin, isoniazide and p-aminosalicylic acid were instituted on the 1st of June During this therapy there

as a transient improvement in her condition The fever ba.ed and she felt better The icterus disappeared Later n however she continued to deteriorate Liver enlarge ient remained cons.ant but the spleen increas d and was inally palpab e three finger breadths below the costal rch There wa no lymphadenopathy Numerous chest ray examinations were negative X ray examination of he gas roin es.inal trac and skeleton in ravenous pyel graphy and cholangiography were normal Th granulo- ytopenia was persistent In June she developed a marked hrombocytopenia After a transient rise in the platelet ount she a ain became thrombocytopenic during July nd the followin. months. The serum alkaline phospha ases were permanently markedly increased and the SGOT nd SGPT levels slightly elevated. There was a successive all in the serum albumin concentration (2 0–1 5 g/100 il) and a moderate increase in the gammaglobulin level

Specimens of gastric lavage stool and fine needle spiration from the spleen taken in May showed growth f uncharacteristic acid fast rods on Lowenstein Jensen nedium at the 4 week reading The guinea pig inocula ons were all negative (Table II) The organism was later lentif'ed as a photochromo enic mycobacterium (Runyon roup I Mycobacterium kansasii) Antibiograms showed esistance to streptomycin, isoniazide and p-aminosalicylic cid These drugs were therefore discontinued and the atient was started on cycloserine and ethionamide on uly 24 The reaction to therapy was unremarkable On August 14 minute pleural effusions were found bilatera.ly nd cultivation of pleural fluid showed growth of Myco- acterium kansasii At the beginning of September small arenchymal infiltrations were seen in the left hilar region ut disappeared on X ray control one week later She had pronounced pancytopenia and required blood transfu ons. The per pheral differential count showed a few netamyelocytes and a few nucleated red cells but was therwise unremarkable Her emaciation was progressive nd she refused further therapy with cycloserine and thionamide Two weeks after discontinued therapy she eveloped high spiking fever and the therapy was re nstituted Her cond tion however became worse she ecame confused suffered from respiratory distress and ed on September 29

Autopsy findings

At autopsy gross examination re ealed marked emacia on. There was 100 ml clear fluid in the right and 300 nl slightly hemorrhagic fluid in the left pleural cavity mall granular fibrin depos ts were seen on the diaphrag atic surfaces of the visceral and parietal pleura on the side The lungs were somewhat small and firm with ematous cut surfaces. No granulomata, infiltrates or ses were found In the abdominal cavity there was ml slightly hemorrhagic ascites but no sign of peri is. The liver was moderately enlarged (16 0 g) but surface was smooth and l ght brown in color No infiltrates were seen. The spleen was greatly enlarged g) soft and of a dark red-brown color The capsule as th ckened and there were fibrinous adhesions to the oneum On the cut surfaces of the spleen many pea grayish soft foci were seen Numerous soft, enlarged nodes up to the size of a marb e were found para aortally in the abdomen, mainly at the level of the superior mesenteric and renal arteries. In some of them necrotic soft centers were seen. An enlarged lymph node with a necrotic center was found in the portal area adjacent to the main bile duct Numerous enlarged lymph nodes up to 1 cm in diameter but without gross necroses were also found in the mediastinum para-aortally and paratrache ally and along the carotid vessels. The bone marrow of the sternum ribs and the spine was grossly normal Red bone marrow was also found in the femur shafts. The remaining organs, including the brain, the genito-urinary and gastrointestinal tracts, were unremarkable

On microscopic examination the *lungs* showed vascular congestion with pulmonary edema. In the left lung small non-specific bronchopneumonic infiltrates were seen and were most numerous n the lower lobe where the pleura on the diaphragmatic surface was covered with f brin deposits No granu omas or acid fast bacilli were found in the lungs. The *liver* showed vascular con.estion and a marked fatty infiltration which wa most pronounced in

Table II *Bacterial findings from clinical specimens*

Date	Specimen	Acid fast organisms on direct microscopy	Growth of acid fast organisms on L-J medium	Guinea pig inoculation
May 5	Urine	–		–
May 13	Urine	N	C	–
May 16	Urine	N	–	–
May 17	Urine	N	–	–
May 19	Gastric lavage	–	+	–
May 22	Endometrical *tissue*	–	–	–
May 23	Gastric lavage	N	C	–
May 24	Stool	N	C	–
May 24	Urine	N	C	–
May 26	Urine	N	–	–
May 27	Gastric lavage	–	–	–
May 27	Stool	N	–	–
May 27	Urine	N	–	–
May 27	Fine needle aspiration from spleen	–	+	–
May 31	Stool	N	C	–
May 31	Urine	N	C	–
June 1	Urine	N	C	–
June 2	Urine	N	–	–
June 14	Stool	N	C	–
July 14	Gastric lavage	N	+	–
July 14	Stool	+	C	–
July 14	Urine		C	–
July 15	Gastric lavage	+	+	–
July 15	Urine		C	–
July 17	Gastric lavage	N	C	–
July 17	Stool	N	C	–
July 18	Stool		C	–
July 22	Stool	–	C	–
Aug 18	Pleural fluid	–	+	–

N = not examined C = contaminated acid fast organisms not found

the periphery of the lobuli Some scattered tiny necroses surrounded by a few plasma cells lymphocytes and macrophages were found No epithelioid cell infiltrates resembling those previously seen in the liver biopsy specimen and no acid fast bacilli were found In the *spleen* several large necroses were seen Most of them were granular containing necrotic cells and cell debris and did not show granulomatous reaction Some of the necroses however were of a caseous structureless type and well demarcated with a fibrous periphery containing epithelioid cells and a few giant cells The latter contained few nuclei and were of a non specific foreign body type Langhans cells were not seen A moderate amount of acid fast bacilli in and around the necroses was found They were large characteristically "beaded" often lying in chains intra as well as extracellularly In the *lymph nodes* from porta hepatis, para aortally in the abdomen and mediastinum and bilaterally from the neck necroses of variable degree were found The necroses were granular with very little granulomatous reaction and without fibrosis Occasional infiltrates of epithelioid cells which were most pronounced in the portal node were found No Langhans cells but some giant cells with the appearance of large histiocytes with a few nuclei were seen In all of the examined lymph nodes many acid fast bacilli were found most numerous in the portal and the abdominal para aortal lymph nodes

The *bone marrow* from sternum ribs lumbar spine and even femur shafts was highly cellular The picture was dominated by the myelopoesis with predominantly immature myeloic cell Megakaryocytes appeared in ordinary amounts. Some large somewhat atypical histiocytes were seen but no evidence was found of hematological malignancy No granulomata and no acid fast bacilli were found

In brain, kidneys small bowel pancreas myocardium adrenal thyroid and hypophysis, nothing pathological was found on histological examination

Culture of spleen liver abdominal and thoracic lymph nodes, ascitic fluid, lungs, pleural fluid brain and cerebrospinal liquor yielded growth of photochromogenic, Runyon group I mycobacteria

Bacteriological and serological findings

The primary isolates as well as subcultures from the different clinical and autopsy specimens all had a colony morphology on Lowenstein Jensen medium characterized by a smooth sometimes confluent growth after 2–6 weeks incubation at 37 C The color was pale beige when the incubation was performed in the dark Subcultures did not show any visible growth after 7 days incubation at room temperature Acid fast staining with Nachtblau according to Hallberg's method revealed on microscopic examination acid fast, somewhat large and often beaded or banded rods with little or no tendency to cord formation

The niacin test according to Peknice and Asmus-Garschagen (11) was negative and the strain was resistant to furan 2-carbonic acid hydrazide at a concentration of 25 μg/ml in Löwenstein Jensen medium (11) Virulence test on guinea pigs performed with 1 mg moist weight bacteria inoculated subcutaneously in the inguinal region was negative at 6-week sacrifice

Sensitivity tests revealed that the strain was resistant to p-aminosalicylic acid, streptomycin, isoniazid and kanamycin, moderately resistant to thiosemicarbazone but sensitive to cycloserine viomycin and ethionamide (resistance ratio values >8 8 >8 8 4 1 1 and 1/8 respectively as compared with H37Rv) Exposure to the light of a 40-watt lamp at a distance of 50 cm for 60 min turned the colonies bright yellow after 12 hours subsequent incubation in the dark. Nitrate reduction test was positive arylsulfatase test negative and the amidase activity pattern was that of a typical Runyon group I bacterium (17)

With respect to the aforementioned findings the strain was classified as a mycobacterium belonging to the photochromogenic Runyon group I species Mycobacterium kansasii. (Dr Ingmar Juhlin at the Department of Bacteriology Malmo General Hospital confirmed this result)

To determine the possible presence of circulating antibodies against mycobacteria a serum sample had been taken in July and was tested according to Ouchterlony's immunodiffusion in gel technique (ID) by means of 17 mycobacterial antigens (concentrated culture filtrates) These antigens represented the following six species M bovis var BCG M kansasii M scrophulaceum, M avium M balnei and M fortuitum The test serum gave positive precipitin reactions with all antigens except those prepared from the species M fortuitum Comparative ID analyses revealed a total of four different precipitins three of which were common to two or more of the mycobacterial species used for the tests. The fourth precipitin was detected only at testing with the M kansasii antigen To determine whether this precipitin could have been produced against a M kansasii specific antigenic factor a comparative analysis with a M kansasii reference precipitation system was performed The reference system used had earlier been well characterized in regard to the immunospecificity of the precipitates forming its pattern. The comparative test revealed that the precipitate formed by this fourth precipitin and the M kansasii antigen coalesced with a precipitate in the reference spectrum labelled *il* The pertinent antigenic factor *i* has so far been demonstrated only in M kansasii strains according to ID analyses of about 250 strains representing the majority of mycobacterial species The finding of the precipitin *I* suggests that the patient was or had been infected with M kansasii One of the aforementioned three precipitins cross-reacting with several mycobacterial species was characterized anti-delta (19) This precipitin has been found in sera from healthy individuals as well as in patients infected with mycobacteria. Immunoglobulin classification of anti-delta from different origins had shown that this precipitin in sera from healthy individuals belongs to the IgM type while that from infected persons may be either of the IgM or IgG type depending on the amount of antigen stimulus and or the duration of disease The anti-delta in the present case was of the IgG type suggesting that this antibody was actually caused by a mycobacterial infection.

Skin test with M kansasii sensitin was never carried out on the patient but her family was tested in March 1968 at a routine dispensary control with the following result PPD kansasii 5 TU husband, age 74 8 × 8 mm son, age 6 negative son, age 3 4 4 mm Chest X ray was negative and tuberculin test positive for all three

COMMENT

Over the past ten years atypical mycobacteria (e g mycobacteria other than M tuberculosis M bovis and M leprae) have been increasingly recognized as causing infection in man Runyon has divided these organisms into four groups on the basis of pigment formation and rate of growth (22) There is a wide regional variation in the species causing human disease The most common site of infection is the lungs Recent reports show that an appreciable number of patients admitted to tuberculosis services have pulmonary lesions due to atypical mycobacteria They usually respond to therapy and have a benign course

Disseminated disease with visceral involvement due to atypical mycobacteria is rare and has usually a fatal outcome (3 8 14 16 18 20 23 24 *25 27 28 30 31 32) The question which* arises in such cases is why the organism usually considered to be of low pathogenicity in man results in a fatal disease It has been presumed that those patients who develop clinical disease have greater susceptibilty In some cases a primary systemic disease such as leukemia might have been the cause of lowered resistance for infection (18) In the present case Mycobacterium kansasii was repeatedly isolated during life and cultured from visceral organs at necropsy Probably in this case too lowered resistance of the host was an important factor in the pathogenesis The cause of the diminished resistance did not seem to be another underlying disease although it might have been attributed to a subclinical infection with Toxoplasma gondii We did not succeed however in isolating the organisms on inoculation Postmortem microscopic examination did not reveal any finding which would indicate the presence of a hematological malignancy or another systemic disease Nor could the treatment with corticosteroids account for the increased susceptibility to infection This treatment was instituted only one week before the first isolation of mycobacteria and the patient had clinical signs of an infectious disease for at least two months before A primary immunological deficiency is unlikely since she had been well before the actual illness

The clinical symptoms were dominated by fever loss and occasionally unproductive cough. Granulocytopenia was an early and persistent laboratory finding Later on thrombocytopenia and marked anemia developed and the pancytopenia became severe during the last months of her illness requiring blood transfusions On many occasions aspiration of sternal marrow proved difficult to perform and yielded only a sparse number of marrow particles *However* histological examinations of biopsies from the spine consistently showed a hypercellular marrow devoid of fat and a delicate augmentation of reticulin fibrils There was however no fibroblastic proliferation of the type seen in primary myelofibrosis The number of megakaryocytes examined on sections was normal Examination of bone marrow smears on different occasions showed a relative increase of erythroid cells and a granulocytic hypoplasia The granulocytopenia was pronounced from the beginning at a time when the spleen was hardly felt on palpation Treatment with prednisolone had no *influence on the granulocyte count The* anemia was due to infection in combination with a hemolytic component Later on the enlargement of the spleen might have been a factor aggravating the anemia The alternative diagnosis in this case presenting fever granulocytopenia and anemia was a malignant granulomatous disease within the reticulo-endothelial organs

It is well established that active disseminated tuberculosis may be associated with a variety of hematological disorders There are many reports of tuberculosis associated with leukemoid reactions and leukemia (10 26) myelofibrosis and polycytemia (1) granulocytopenia thrombocytopenia and pancytopenia (4 7 9) In some cases hypersplenism has been suggested as a mechanism of pancytopenia In many cases tuberculosis seems to be a complicating infection in a primary hematological disorder in others especially in the pancytopenias disseminated tuberculosis seems to be the primary disease However not enough attention has been directed to the association of blood dyscrasias accompanied by disseminated infections with atypical mycobacteria Hitherto twenty two cases of disseminated visceral infection with atypical mycobacteria have been reported Nine of them were due to the photochromogenic group I (3 14 20 30 32) eleven to the non photochromogenic group III (8 16 18 23 24 25 27 28 31) and two to the scotochromogenic group II type of mycobacteria (18 32) A comparison between the clinical data reported for patients with the group I type of infection on the one hand and the group III type on the other suggests that there

might be differences in the clinical picture of these two groups Nine of the eleven patients infected with the group III mycobacteria were children while all but one of the nine cases infected with the group I mycobacteria were adults All patients in both groups in whom blood values were reported had anemia and many had a severe anemia which required blood transfusions There was however a striking difference as regards the white blood cell count In the seven cases with group III mycobacterial infection whose white cell count was stated all but one had a pronounced leucocytosis In four cases the count was above 30 000 associated with a moderate granulocytic immaturity and these were judged to be leukemoid reactions In one case of group II infection reported by McCusker and Green there was a white cell count of 155 000/mm³ with numerous immature myeloic cells (blasts promyelocytes and myelocytes) in the peripheral blood In this case a diagnosis of chronic granulocytic leukemia was made and the patient received cytostatic therapy six months prior to the first suggestion of a mycobacterial infection (18) It is possible that in this case mycobacterial infection was a superposition At autopsy however no leukemic infiltrates were found in other organs than the bone marrow On the other hand in the patients infected with the group I mycobacteria six of seven for whom values were reported had a pronounced granulocytopenia Thrombocytopenia was a finding in three cases of group III infection and in all five cases of group I for whom the platelet count was stated The platelet count was usually normal on admission but like the present case the count decreased with worsening of the patient s condition (14 16 32) The bone marrow was usually cellular or hypercellular in infections due to group III mycobacteria In the cases infected with group I mycobacteria granulocytic hypoplasia was found in three cases (14 32) bone marrow sections showed a normal cellularity in three cases (14 32) and hypocellularity in one case (32) As in the present case aspirated bone marrow smears showed in some cases a hypocellularity but bone marrow sections when examined appeared to be hypercellular (14 32) Granulomata in the bone marrow in the presence and absence of acid fast bacilli were more often found in group III and group II infections but only in one case infected with the group I mycobacterium Bone lesions were also more often reported in disseminated infection with group III mycobacteria and all of them were recorded in children. The skeletal lesions in the children in some cases gave suspicion of reticulo-endothelioses Miliary infiltrates in the lungs on chest X ray examination were occasionally found in both types of infection but many were normal or uncharacteristic The tuberculin skin tests were negative in all but one case insofar as these tests have been reported Skin eruptions have been reported in several cases (16 28 32) Skin ulcerations with underlying bone lesions were reported in the group III type of infection (25 27)

The patho-anatomical findings in the present case showed necrotic lesions in lymph nodes and spleen with little granulomatous reaction as described earlier in generalized disease due to M tuberculosis (2) and photochromogenes (3) A characteristic feature was the presence of numerous large beaded bacteria The demonstration of such bacteria seems to be the only diagnostic criterion also in chronic granulomatous lesions due to group I mycobacteria as these lesions exhibit all the histopathologic features seen in infections with M tuberculosis (13) The suspicion of a granulomatous disease derived in the present case from a liver biopsy The diagnosis of group I mycobacteriosis was however due to bacteriological procedures

Most of the hitherto reported cases of disseminated infection with atypical mycobacteria had a fatal outcome despite therapy However some although short lived effect of antituberculous therapy could be observed (25 32) Remissions after effective drug therapy were followed by relapses probably due to the emergence of drug resistance It has been pointed out (25) that in disseminated infections with poor immune response it is wise to give at least four drugs together especially in the initial phase of treatment The drugs worth trial include streptomycin erythromycin ethambutol and cycloserine Early diagnosis and laboratory tests of drug efficacy are important

Hematological dyscrasias and hepato-splenomegaly are common findings in cases of disseminated mycobacterial infection It should be stressed that disseminated infection with atypical mycobacteria may be the cause of a variety of hematological disorders in the same way as dissemination with M tuberculosis On the other hand infections with atypical mycobacteria may be super

imposed on primary hematological disorders and other systemic diseases with lowered resistance of the host

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AN IN VITRO DEMONSTRATION OF CELLULAR IMMUNITY AGAINST AUTOLOGOUS MAMMARY CARCINOMA IN MAN

PRELIMINARY REPORT

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Abstract In five out of eight patients operated on for mammary carcinoma, an extract of the autologous tumour induced inhibition of the in vitro leukocyte migration. Extracts of the patient s normal mammary tissue gave no inhib tion and negative results were likewise obtained when leukocytes from matched controls were exposed to extracts of isogenei normal or tumourous tissue These observations suggest that in some patients mammary car cinoma is associated with a state of cellular hypersensi ti ity against components of the tumour

Very few data concerning cellular immunity against autologous tumours in humans are avail able (5) Hughes and Lytton (3) employed intra cutaneous injection of extract of autologous tu mour using as control extract of corresponding normal tissue from the same patient They found a positive delayed hypersensitivity response with a negative control in 11 out of 33 patients 16 had mammary carcinoma three of whom showed a definite positive response whereas in two a weak positive reaction was obtained

Hellstrom et al (2) demonstrated that the growth of autologous neuroblastoma cells in vitro was inhibited by lymphocytes from children who were either cured of neuroblastoma or who had per sistent tumours whereas the growth of autologous skin fibroblasts was not inhibited This selective inhibition was also seen with lymphocytes from other children with neuroblastoma and from four of five mothers whose children had neuroblastoma Lymphocytes from a variety of control patients including children with other malignant tumours were uniformly negative

In this communication the preliminary results of an in vitro analysis of the response of leuko-cytes from patients with mammary carcinoma to an extract of their own tumour are reported The system employed is the leukocyte migration tech nique (1) Evidence has been presented (6) that in this test system an antigen induced inhibition of the migration is a correlate to cellular (delayed type) hypersensitivity If mammary carcinoma is associated with a state of cellular hypersensitivity directed towards antigenic tumour components this might be revealed by the technique employed

MATERIAL AND METHODS

Eight conse utive female patients with mammary carcinoma constitute the clinical material (Table I) None of the pa tients had clinical evidence of metastases or of local inflammatory reaction At operation the tumour was excised in toto A portion was taken for histological examinat on, and the tumour was then separated under aseptic conditions at the same time a part of the glan dular tissue which was not macroscopically invaded by the tumour was obtained These specimens were cut into small pieces suspended in Hanks solution and homo genized in a M S E homogeniser for 5 min The homo genates were kept at 4 C overnight and then centrifuged at 1000 g for 20 min The protein concentration of the supernatant was adjusted to 1 mg per ml by dilution with Hanks solution

Mammary glandular tissue obtained at autopsy from patients without malignant d sease was prepared n a s milar fashion.

The tumours were classif ed histologically according to criteria d scussed previously (4)

The leukocyte m gration test was performed as descr bed by Bend xen and Søborg (1) In pilot experiments the highest concentration of extract not causing unspecific inh b tion of migration was 100 μg protein per ml culture med um, and this dose was used throughout

On the day following mastectomy blood was

Table I. *Data on eight patients with mammary carcinoma*

		Histology of tumour				Migration index[a]					
						Patient			Control		
Pat.	Age	Histological type	Grade of anaplasia[b]	Lymphocytic infiltration (0 – +++)	Necrosis (0 – +++)	PTu	PGlTi	AuGlTi	PTu	PGlTi	AuGlTi
1	70	Invasive duct carcinoma	III	(+)	0	0.91	1.21	1.02	1.18	1.13	0.95
2	70	Invasive duct carcinoma	II	(+)	0	0.74	0.86	0.89	0.90	0.91	0.89
3	70	Invasive duct carcinoma	II	0	0	0.63[c]	1.04	0.95	0.97	1.36	0.97
4	71	Invasive duct carcinoma	II	+	0	0.79[c]	1.06	0.94	1.02	1.01	1.04
5	46	Partly lobular carcinoma	II	0	0	0.73[c]	1.05	0.99	0.90	0.97	0.95
6	68	Invasive duct carcinoma	II	+	0	1.10	1.05	1.06	1.12	1.14	1.09
7	45	Invasive duct carcinoma	III	++	+	0.74[c]	1.00	1.02	1.04	0.94	1.00
8	51	Invasive duct carcinoma	III	+	0	0.97	0.98	0.97	0.94	0.95	0.94

[a] The results of the leukocyte migration test are expressed as Migration index (see text). As antigens were used extracts of patient's mammary tumour (PTu), patient's non-tumourous mammary glandular tissue (PGlTi), mammary tissue obtained at autopsy from patients without malignancy (AuGlTi).
[b] Grading of anaplasia: I low, II medium, III high.
[c] Cultures showing inhibition.

heparin. After sedimentation the leukocytes were washed 4 times in Hanks' solution and collected in capillary tubes. Each of these was placed in a culture chamber containing 1.0 ml of TC 199 with 10% horse serum. To the test cultures 0.1 ml tissue extract containing 100 μg protein was added. All cultures were set up in quadruplicate. After 24 hours the migration area of leukocytes around the opening of the capillary tube was measured in a projection microscope. Within a set of identical cultures the variation from one migration to another did not exceed 10%. The average migration area of extract-containing (Mx) and control (Mo) cultures from the same blood sample determines the migration index (MI):

$$MI = \frac{Mx}{Mo}$$

A value of less than 1.0 thus indicates an inhibition of migration. For each patient examined a control patient without malignant disease, matched for age and sex, was studied in parallel series of cultures.

RESULTS

The results obtained in the eight patients and their matched controls are summarized in Table I. In cultures from the control patients the addition of mammary extract (100 μg protein) from both tumourous and normal tissue gave no inhibition of leukocyte migration, the values for MI ranging from 0.89 to 1.36, with a mean of 1.01. In five of the patients with mammary carcinoma an inhibition of leukocyte migration (MI below 0.80) was induced by the tumour extract. In no instance was inhibition induced by extract of the patient's own non-tumourous mammary tissue or by extract of isogeneic mammary tissue obtained at autopsy.

DISCUSSION

It is of interest to compare the two in vitro techniques by which a state of cellular immunity against autologous malignant tissue has been demonstrated in man: the colony inhibition assay of Hellström et al. (2) and the migration inhibition test described in the present report. In colony

inhibition cytotoxicity of sensitized lymphocytes against target cells is demonstrated With this technique the question of necrotic antigens i e acquisition of new antigens solely due to necrosis in the tumour does not arise This source of error cannot be excluded in the migration inhibition test On the other hand this technique is open to much wider applications since it does not depend on the availability of living target cells in culture

The application of such a technically simple in vitro technique for the detection of cellular anti tumour immunity in man seems to offer great possibilities A larger series is necessary in order to investigate the relationship between histological appearance and presence or absence of immunity Also investigations of pre malignant conditions and non malignant states with mammary necrosis may prove of interest Follow up studies of the individual patients can establish whether a positive immunological response influences the prognosis The future development of the reaction must be followed both in patients in whom the treatment proves radical and in those who develop metastases it will be of interest to establish whether the immune reactivity disappears in patients who are overwhelmed by their tumour

Examination of the specificity of the response will reveal whether patients react exclusively with their own tumour or whether cross reactions with extracts of other tumours can be demonstrated—the latter possibility corresponding to tumours induced in syngeneic animals by the same virus as opposed to the immunological uniqueness of tumours induced by the same chemical carcinogen

By means of this technique it seems feasible to characterize the antigens responsible for the reaction with regard both to their subcellular localization and their biochemical nature

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HAEMODYNAMIC EFFECTS OF TRASICOR®, A NEW BETA ADRENERGIC BLOCKING AGENT, IN HYPERTENSIVE PATIENTS

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Abstract Five hypertensive patients with left ventricular hypertrophy and minimal pulmonary signs of left sided heart failure were treated for five weeks with oral Trasicor®, a new beta adrenergic blocking agent. During the course of the treatment the basal arterial pressure in the upright position declined very slightly. Haemodynamic studies were performed at rest and during submaximal supine exercise before and during beta blockade. Negative chronotropic action on the heart was obvious. Whether the drug had any negative or positive inotropic effects on the heart remained uncertain because of inconsistent haemodynamic changes and because of the relatively profound reflex and autoregulatory alterations in left ventricular performance induced by exercise.

Recently several new drugs thought to act as beta adrenergic blocking agents have been developed. Increased interest in finding new compounds of this sort has been due to the probable clinical usefulness of beta adrenergic blockade as well as to the need to develop a drug with minimal depressing effect on myocardial contractility yet with properties of a beta blocker.

Results concerning the antihypertensive effect of the beta adrenergic blocking agents have been inconsistent (3, 5–9, 11–13) and therefore the therapeutic value of these drugs in arterial hypertension is still debatable. In the present paper we report the haemodynamic effects of prolonged peroral administration of a new beta adrenergic blocking agent, Trasicor®, in patients suffering from essential hypertension with left ventricular hypertrophy. The haemodynamic studies were done at rest and during submaximal exercise.

MATERIAL AND METHODS

Five patients, two females and three males, aged 43–53 years, volunteered for the study. The patients were selected in the outpatient clinic on grounds of radiological and/or electrocardiographic evidence of left ventricular hypertrophy with minimal or absent signs of left ventricular failure. The duration of diagnosed hypertension in the patients was 1–15 years. Their disease was classified as essential hypertension by exclusion of renal and endocrinological causes of hypertension: urinary sediment, urinary protein excretion, endogenous creatinine clearance, intravenous urography, serum sodium, potassium and pH and urinary excretions of vanillylmandelic acid and metanephrines were all normal. Coronary and valvular heart diseases were also excluded.

The patients were admitted to hospital for haemodynamic studies and initiation of the treatment. Previous antihypertensive medication was withheld two weeks before hospitalization and throughout the trial, and no drugs were allowed except the one to be tested. During the first week in hospital (the third week without any treatment) the arterial pressure was measured in the upright position every morning with a sphygmomanometer and at the end of the week the first haemodynamic study, to obtain the initial values, was performed. Thereafter treatment with Trasicor® (1-(o-allyloxyphenoxy)-3-isopropylamino-2-propanol hydrochloride) was initiated (Trasicor® was supplied from Ciba AG, Basel). The initial dosage, given as four divided doses per os, was 160 mg daily for one week; in the 2nd week the dosage was increased to 240 mg daily, in the 3rd week to 320 mg daily and in the 4th week to 400 mg daily. The patients were discharged in the 2nd week of treatment, studied twice a week in the outpatient clinic for possible untoward symptoms or signs, and admitted to hospital at the end of the 4th week of treatment. The daily dosage of 400 mg was continued for another week in hospital and upright arterial pressure was measured daily. At the end of the 5th week of treatment the haemodynamic study was repeated.

The haemodynamic studies were performed similarly before and during treatment with 400 mg Trasicor® daily in the morning and while the patients were in a fasting state. The pressures in the pulmonary artery and in the pulmonary capillary wedged position were measured by right heart catheterization and the pressures in the brachial artery by puncture and cannulation with a teflon catheter. The pressure pulses were recorded utilizing a Sanborn

Table I *Haemodynamic data at rest and during exercise before and during treatment with Trasicor*®

	Pat 1		Pat 2		Pat 3		Pat 4		Pat 5	
	Before	During	Before	During	Before	During	Before	During	Before	During
Heart rate (beats min) at rest	84	70	63	58	98	81	86	78	86	66
during exercise	140	120	105	94	16	132	138	1?5	14?	1?0
Cardiac output (l min) at rest	7 4	6 4	6 5	5 0	10 5	4 7	4 0	3 9	6 4	6 0
during exercise	17 5	15 6	11 1	10 1	13 6	8 1	10 2	7 [illegible]	13 6	13 8
Stroke volume (ml) at rest	88	91	103	85	106	59	46	50	74	91
during exercise	?25	130	106	107	85	61	74	58	96	115
Mean brachial artery pressure (mm Hg) at rest	190	163	140	149	144	156	160	180	160	176
during exercise	*160*	*14*	*160*	*157*	*156*	*18?*	*185*	*[illegible]00*	*194*	*?08*
Systemic vascular resistance (units) at rest	26	25	?1	29	13	33	39	45	[illegible]4	?9
during exercise	9	9	14	15	11	22	18	?8	14	15
Mean pulmonary capillary wedge pressure (mm Hg) at rest	11	11	8	8	10	5	10	1[illegible]	8	9
during exercise	14	0	?4	24	17	11	11	10	1?	1?
Mean pulmonary artery pressure (mm Hg) at rest	21	20	16	16	16	17	17	23	16	—
during exercise	—	9	28	34	26	25	—	1?	?0	—
Pulmonary vascular resistance (units) at rest	1 4	1 4	1 2	1 6	0 6	? 6	1 8	2 8	1 2	—
during exercise	—	0 6	0 7	1 0	0 7	1 6	—	0 3	0 6	—
LV ejection time (sec) at rest	*0 30*	*0 32*	*0 27*	*0 28*	*0 25*	*0 25*	*0 [illegible]8*	*0 30*	*0 ?6*	*0 30*
during exercise	0 24	0 ?7	0 ?3	0 25	0 2[illegible]	0 [illegible]3	0 [illegible]2	0 25	0 2[illegible]	0 ?7
LV mean systolic ejection rate (ml sec) at rest	290	[illegible]85	380	304	480	236	164	167	290	304
during exercise	5 0	481	460	4?8	386	66	336	3[illegible]	436	4 5
LV minute work (kpm min) at rest	20 2	15 0	13 1	10 7	21 7	10 5	9 ?	10 1	14 7	15 2
during exercise	40 1	31 8	25 4	22 8	30 4	?1 2	27 1	?0 6	37 8	41 1
LV stroke work (kpm) at rest	0 4	0 21	0 21	0 18	0 22	0 13	0 11	0 13	0 17	0 3
during exercise	0 29	0 ?7	0 ?4	0 24	0 0	0 16	0 0	0 17	0 27	0 34
Rate pressure product (units) at rest	227	164	126	127	196	16	185	197	172	166
during exercise	30	188	2[illegible]0	183	300	?67	[illegible]34	82	296	311

pressure transducer (model 67B) with optical galvanometers on photographic paper at a speed of 50 mm sec First the pressures at rest in the pulmonary artery pulmonary capillary wedged position and brachial artery the heart rate and the cardiac output using the Fick prin-

Table II *Systemic arterial blood pressure before and after 5 weeks treatment with oral Trasicor*®

Pat	Arterial pressure in the upright position as measured by sphygmomanometer		Intra-arterial pressure during cardiac catheterization at rest	
	Before	During	Before	During
1	00 100	195 80	70 1?0	[illegible]34 11
2	205/1 0	175 105	?00 115	19 116
3	175/115	155 100	00 11	?00 1?0
4	2 0 1 5	190 110	15 1 0	[illegible]53 140
5	2[illegible]5 130	??0 115	?00 110	[illegible]5 *134*

ciple were determined with the patient in the supine position with his lower extremities already connected to a bicycle ergometer to achieve the same initial position as during exercise Thereafter exercise was started first with a load of 150 kpm min for two minutes to get the patient accustomed to the procedure and then with a load of 300 kpm min which was maintained for at least four minutes, before the same haemodynamic measurements [illegible] at rest were repeated while the patient was still exer[illegible]

The following variables were calculated from the ha[illegible] modynamic data systemi and pulmonary vascular r[illegible] s[illegible]tance stroke volume left ventricular ejection time ([illegible] a mean of five consecuti e beats from brachial arter[illegible] pressure cur[illegible]s) mean systolic ejection rate left [illegible] ular minute work and stroke work, and the rate p[illegible] product of systemic circulation as defined by R[illegible] (10)

The chest rad ographs were taken before and dur[illegible] th[illegible] treatment without reference to the cardiac cycle The vo[illegible] ume of the heart, the relati e sizes of its chambers a[illegible] of the large blood vessels, and the radi[illegible] ogical s[illegible] of left ventricular failure were estimated by the meth[illegible] described previously from this laboratory (4)

Table III *Findings in the chest radiographs before and during treatment with Trasicor®*

	Pat	Heart volume		Grade of enlargement		Left ventricular failure
		Absolute (ml)	Relative (ml m² BSA)	Left atrium	Left ventricle	
B fore	1	1000	550	1	1	1a
During		930	540	1	1	1b
Before	2	785	485	1	0	1a
During		1100	670	2	1	1a
Before	3	710	410	1	1	1b
During		650	375	1	1	1b
Before	4	1160	590	2	1	1a
During		1140	580	2	1	2
Before	5	1000	540	1	1	1a
During		1090	575	1	1	1a

Left at ium
0 = normal
1 = slight but no longer normal indentation in the lateral view of the oesophagus
2 = distinct displacement of the oesophagus

Left vent icle
0 = normal
1 = slight rounding of the apex
2 = distinct enlargement and displacement downward or outward

Left ventr cular failu e
0 = normal
1a = venod latation in the lower and middle fields and/or slight hilar or pulmonary clouding
1b = marked venodilatation in all the pulmonary fields and/or distinct clouding
2 = venoconstriction in the lower fields attended by marked venodilatation in the upper fields
3 = distinct alveolar pulmonary oedema

RESULTS

The results are given in Tables I II and III and in Fig 1

Heart rate

The treatment with Trasicor® decreased the heart rate at rest as well as the heart rate response to the same work load At rest the heart rate was 5–20 beats slower (a mean of 15% less than the pretreatment values) and during exercise corre spondingly 11–30 beats (mean 13) slower during treatment than before it. However the relative ase in heart rate in response to the same work load was similar both before and during treatment

Cardiac output

At rest the cardiac output was always less during tment than before it, the decline varying be ween 0 1 and 5 8 l/min (mean 20 %) During the increase of cardiac output remained during treatment than before it the difference ying between 1 0 and 5 5 l/min except in one ent (pat 5) whose exercising cardiac output was unchanged (mean decrease 18 %) However the relative increase of cardiac output in response to exercise was similar both before and during treatment

Stroke volume

At rest no constant change in stroke volume was induced by treatment During exercise the stroke volumes usually increased slightly but no constant difference was found between exercising stroke volumes before and during treatment

Brachial artery pressure

Arterial pressure calculated as a mean of the sphygmomanometer measurements on seven morn ings in hospital before and during treatment showed a decrease in all patients during treat ment (Table II) The systolic pressure declined by 5–30 mm Hg (mean 9%) and the diastolic pres sure by 15–20 mm Hg except in one patient whose mean diastolic pressure rose by 5 mm Hg (mean d crease 10) The direct intra arterial pressures measured during cardiac catheterization

Table I *Haemodynamic data at rest and during exercise before and during treatment with Trasicor®*

	Pat 1		Pat 2		Pat 3		Pat 4		Pat 5	
	Before	During	Before	During	Before	During	Before	During	Before	During
Heart rate (beats min) at rest	84	70	63	58	98	81	86	78	86	66
during exercise	140	1 0	105	94	16	132	138	125	14?	1?0
Cardiac output (l min) at rest	7 4	6 4	6 5	5 0	10 5	4 7	4 0	3 9	6 4	6 0
during exercise	17 5	15 6	11 1	10 1	13 6	8 1	10 2	7 2	13 6	13 8
Stroke volume (ml) at rest	88	91	103	85	106	59	46	50	74	91
during exercise	125	130	106	107	85	61	74	58	96	115
Mean brachial artery pressure (mm Hg) at rest	190	163	140	149	144	156	160	180	160	176
during exercise	160	142	160	157	156	18?	185	?00	194	?08
Systemic vascular resistance (units) at rest	26	25	21	?9	13	33	39	45	?4	?9
during exercise	9	9	14	15	11	2?	18	28	14	15
Mean pulmonary capillary wedge pressure (mm Hg) at rest	11	11	8	8	10	5	10	12	8	9
during exercise	14	20	24	24	17	11	11	10	12	12
Mean pulmonary artery pressure (mm Hg) at rest	?1	20	16	16	16	17	17	23	16	—
during exercise	—	?9	?8	34	?6	25	—	1	0	—
Pulmonary vascular resistance (units) at rest	1 4	1 4	1 2	1 6	0 6	2 6	1 8	2 8	1 2	—
during exercise	—	0 6	0 7	1 0	0 7	1 6	—	0 3	0 6	—
LV ejection time (sec) at rest	0 30	0 32	0 27	0 28	0 25	0 25	0 28	0 30	0 6	0 30
during exercise	0 24	0 27	0 23	0 25	0 22	0 23	0 22	0 25	0 ??	0 ?7
LV mean systolic ejection rate (ml sec) at rest	?90	2?5	380	304	480	236	164	167	290	304
during exercise	520	481	460	428	386	266	336	232	436	425
LV minute work (kpm min) at rest	?0 2	15 0	13 1	10 7	21 7	10 5	9 2	10 1	14 7	15 ?
during exercise	40 1	31 8	25 4	2? 8	30 4	21 2	27 1	20 6	37 8	41 1
LV stroke work (kpm) at rest	0 24	0 21	0 21	0 18	0 ?2	0 13	0 11	0 13	0 17	0 ?3
during exercise	0 ?9	0 27	0 ?4	0 ?4	0 ?0	0 16	0 20	0 17	0 ?7	0 34
Rate pressure product (units) at rest	?7	164	126	127	196	16?	185	197	17?	166
during exercise	30	188	? 0	183	300	?67	274	282	296	311

pres ure transducer (model 67 B) with optical galvanometers on photographic paper at a speed of ?0 mm sec First the pressures at rest in the pulmonary artery pulmonary capillary wedged position and brachial artery the heart rate and the cardiac output using the Fick principle were determined with the patient in the sup... position, with his lower extremities already connected to bicycle ergometer to achieve the same initial position during exercise Thereafter exercise was started first w... a load of 1?0 kpm/min for two minutes to get the p... tient accustomed to the procedure and then with a l... of 300 kpm min which was maintained for at least f... minutes, before the same ha modynamic measurements at rest were repeated while the patient was still exercis...

The following variables were calculated from the h... modynamic data systemic and pulmonary vascular r... sistance stroke volume left ventricular ejection time (... a mean of fi e consecutive beats from brachial arte... pressure curves) mean systolic ejection rate left ventr... ular minute work and stroke work and the rate press... product of systemi circulation as defined by Robins... (10)

The chest rad ographs were taken before and during t... treatment without reference to the cardiac cycle Th vo... ume of the heart, the relative sizes of its chambers a... of the large blood vessels, and the radiological signs ... left ventricular failure were estimated by the metho... described previously from this laboratory (4)

Table II *Systemic arterial blood pressure before and after 5 weeks treatment with oral Trasicor®*

Pat.	Arterial pressure in the upright position as measured by sphygmomanometer		Intra arterial pressure during cardiac catheterization at rest	
	Before	During	Before	During
1	?00 100	195 80	?0 1 0	?34 112
2	205 1?0	175 105	?00 115	?19 116
3	175 115	155/100	00 11?	?00 1?0
4	?20 1?5	190 130	215 1 0	253 140
5	2?5 130	220 115	200 130	?5? 134

Mean pulmonary capillary wedge pressure

No significant change in the resting mean PCW pressures was induced by the treatment. During exercise without treatment the mean PCW pressure increased in all by 1–16 mm Hg. During treatment the pressures increased to the same extent as without treatment in individual patients. One patient (pat. 2) showed a mean exercising PCW pressure of 24 mm Hg both before and during treatment.

Pulmonary artery mean pressure and vascular resistance

No consistent changes were observed in the cases in which measurements were performed.

Left ventricular ejection time

At rest the left ventricular ejection time increased in four patients and remained the same in one patient, roughly corresponding to the changes in heart rate during treatment. During exercise the ejection time was similarly always longer during treatment than before it (mean increase 13%). The change could be ascribed to the slower exercising heart rate during treatment. The relative mean shortening of the exercising ejection time was slightly less during treatment (12%) than before it (17%).

Mean rate of left ventricular ejection

At rest no constant change in the mean rate of left ventricular ejection was observed during treatment. During exercise the mean ejection rate was always less during treatment than before it, the difference varying from 11 to 120 ml/sec (mean 16%). The relative mean increase of the mean exercising ejection rate was slightly less during treatment (40%) than before it (47%).

Left ventricular work indices

Left ventricular minute work and stroke work at rest did not show constant changes during treatment; a decrease was observed in three patients and a slight increase in two others. During exercise the left ventricular minute work was less during treatment than before it in all but one patient (mean decrease 15%). Exercising stroke work did not change in a constant pattern during treatment. The rate pressure product, which it has been suggested reflects internal left ventricular work (10), seemed to change in a random pattern both at rest and while exercising during treatment.

Radiological findings

The heart volume remained the same in all but one patient (pat. 2) whose relative heart volume increased from 485 to 670 ml/m² BSA. Radiological pulmonary signs of left ventricular failure progressed in another patient (pat. 4). In the other patients no changes in radiological signs were seen.

Side-effects of the drug

No annoying symptoms or signs of toxicity were observed during the treatment.

DISCUSSION

The antihypertensive effect of five weeks peroral treatment with the new beta adrenergic blocking agent, Trasicor®, is slight, according to the present results. The systemic arterial pressure in the upright position declined in all the patients during treatment but not to recommendable normotensive levels. However, direct intra-arterial measurements during cardiac catheterization showed an increase rather than a decrease of arterial pressure during treatment. This discrepancy may reasonably be explained by the combined effect of supine position at catheterization, the casual nature of the intra-arterial measurement, and the psychic stress of the catheterization procedure. The information in the recent literature on the immediate hypotensive effects of intravenously administered beta adrenergic blocking agent is not for the most part comparable with data such as ours obtained during prolonged peroral treatment. At present this beta adrenergic blocking agent does not seem to have much practical value in the treatment of essential arterial hypertension.

The negative chronotropic effect of Trasicor® on the resting heart rate and on the response of the heart rate to exercise was obvious.

During the treatment the cardiac output at rest and while exercising was somewhat less than without the drug, suggesting a negative inotropic action or simply a secondary influence on the heart rate response. In fact, the stroke volumes and the pulmonary capillary wedge pressures at rest and during exercise remained the same despite the drug, indicating that the left ventricle was capable of the same stroke volume with the same filling

pressure while the heart volumes remained unchanged radiologically Thus no remarkable depression of myocardial contractility had occurred With the methodical errors involved the changes of left ventricular ejection times and mean rates of left ventricular ejection while exercising during treatment do not seem significantly different from the pretreatment values Left ventricular minute work parallelled the cardiac output during treatment and stroke work parallelled the stroke volumes as no remarkable changes in systemic vascular resistance occurred Thus none of our haemodynamic findings show a definite negative or positive inotropic effect of Trasicor®

On the other hand it is worth noticing that in the intact circulation even with increased left ventricular afterload and myocardial hypertrophy such as our patients had the immediate reflex and autoregulatory adjustments of myocardial contractility induced by exercise may well mask any negative inotropic effects of a drug especially in the supine position (2) The most important of these regulatory factors influencing the normal cardiac response to exercise are increase in heart rate sympathetic drive and operation of the Frank Starling mechanism each of which may also operate independently according to the degree of load applied (1) During supine submaximal exercise the requirements of tissue perfusion are satisfied mainly by an increase in the heart rate provided the heart is not working on the declining part of the ventricular function curve

Since the introduction of the beta adrenergic blocking agents caution has been practised in their use in heart failure because of their negative inotropic effects In the present study no definite haemodynamic signs of impaired performance of hypertrophic left ventricles were observed during prolonged treatment with Trasicor® at rest or during submaximal supine exercise It seems likely that in daily doses of up to 400 mg orally Trasicor® has no definite injurious effect on the function of a competent hypertrophic left ventricle

ACKNOWLEDGEMENT

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ADAMS-STOKES SEIZURES IN PATIENTS WITH ATTACKS OF BOTH TACHY- AND BRADYCARDIA A THERAPEUTICAL CHALLENGE

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Abstract The case histories of six patients with Adams-Stokes seizures and attacks of tachy and bradycardia are reported Two patients were managed by pacemaker implantation alone while three patients had to be treated with the combination of pacemaker and a beta receptor blocking drug. The necessity of continuous ECG supervision for diagnosis of the arrhythmias, for selection of proper therapy and control during therapeutical trials is stressed

The introduction of tape recording in clinical electrocardiography and the establishment of more or less automatic methods for rapid analysis of the ECG recorded on tape have made it possible to follow changes in heart rhythm during extended periods of time Such techniques have already proved helpful in elucidating problems concerning the clinical importance of paroxysmal arrhythmias and the causal relation between such arrhythmias and sudden spells of dizziness and fainting It is the purpose of this paper to draw attention to a small group of patients complaining of episodes of both palpitation and fainting in whom long term ECG supervision revealed paroxysms not only of tachycardia but also of asystole caused by transitory atrio-ventricular or sino-atrial block From a therapeutical point of view these patients present a real tricky problem as an effective drug treatment of their tachycardias may increase their tendency to develop conduction disturbances whereas an effective drug prophylaxis of their episodes of block may result in an aggravation of their tendency to develop tachycardias and may be ventricular fibrillation

MATERIAL

This series comprises six patients, two men and four women aged 5–69 years (Table I) Three of these patients showed definite signs of coronary artery disease either in their histories or by changes in their ECG and one had rheumatic heart disease whereas the etiology of the heart disease remained obscure in the last two patients. All six patients had had attacks of palpitation and dizziness or fainting for lengthy periods, i.e from three months to twenty years.

CASE REPORTS

Case 1

Female born 1916 She was admitted to our department in 1964 For two years she had had increasingly severe attacks of tachycardia often provoked by exercise In between she also had dizzy spells. Various antiarrhythmic drugs had been tried without any success. Her ECG at rest showed a sinus bradycardia at about 50/min (Fig. 1) A bicycle ergometer test (400 kpm for 1 min 600 kpm for 2 min) provoked ventricular ectopics which progressed to ventricular tachycardia (Fig 1) Long-term ECG recording also revealed periods of sino-auricular block sometimes causing fainting, whereas fainting during an attack of tachycardia was rare Apart from a moderately enlarged heart (720 ml/m²) and the arrhythmias mentioned, no other signs of cardiac disease were found. The patient's functional capacity was severely restricted

In December 1964 treatment with propranolol was started. The tachycardia was well controlled, but the dizzy spells became more frequent. So the dosage was reduced from 1_0 mg per day to 70 mg per day In March 1966 the tachycardia reappeared, alternating with sino-auricular block or extreme bradycardia and often causing dizziness or fainting (Fig) Discontinuation of propranolol for one month did not alter the situation Treatment with procainamide 1 g per day diphenylhydantoin 300 mg per day and diphenylhydantoin in combination with atropine did not prevent attacks of tachycardia.

It was concluded that propranolol should be given in bigger dosages. However anticipating an aggravation of the spontaneously occurring sino-auricular block which already troubled the patient very much, we decided to implant a fixed rate epicardial pacemaker first. This effectively controlled the bradycardia, and propranolol 1 0 mg per day prevented tachycardia even during exercise

Table 1 *Age sex and type of heart disease in six patients with alternating tachycardia and block*

Case no	Year of birth	Age	Sex	Type of heart disease
1	1916	5?	♀	Unknown
2	1915	53	o	Ischemic
3	191?	56		Rheumatic
4	1905	63	♂	Unknown
5	190?	66	♀	Ischemic
6	1899	69	♀	Ischemi

(This course of events has been described in an earlier publication (6))

The patient was seen again in March 1968 i e two years after pacemaker implantation She had had no attacks of tachycard a or fainting but complained of palpitations especially during exercise This turned out to be due to interference between the patient s own heart action and the artificial pacemaker For this reason the fixed rate pacemaker was replaced by a demand pacemaker Since then the patient has been fit and well on treatment with propranolol 90 mg per day During a recently performed exercise test there was no ectopic activity whatsoever (Fig 3)

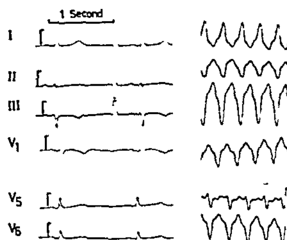


Fig 1 ECG of case 1 during her first admission in 1964 Left sinus bradycardia at rest Right ventricular tachycardia immediately after a bicycle ergometer test (?00 kpm for 1 min 600 kpm for 2 min)

Case

Male born 1915 This man was admitted to a local hospital on November 19 1967 because of acute myocardial infarction of the anterior wall On the second day in hospital he got an embolus in his left femoral artery The course was also complicated by long lasting hypotension. In periods he had a rather pronounced ectopi acti ity After about three weeks he started to get recurrent attacks of tachycardia and fainting This was the reason for his being transferred to our coronary unit on December 16 1967 On arrival he was in sinus rhythm The ECG showed signs of recent anterior wall infarction and a few supraventricular ectopic beats On the first night he suddenly developed ventricular tachycardia which abruptly changed into asystole of 14 sec duration before sinus rhythm recurred (Fig. 4) Anti arrhythmic treatment seemed necessary but because of the asystole occurring during his attack of tachycardia a pacemaker catheter was first introduced into his right ventricle During positioning of the catheter he had several attacks of ventricular tachycardia and fibrillation which had to be stopped by DC shock. Treatment with propranolol in increasing dosages (up to 100 mg per day) was initiated During the next week no further arrhythmias occurred and the treatment was discontinued During the next two weeks he had a varying number of extrasystoles (up to .0%) most of which were supraventricular Besides he had short lasting attacks of tachycardia passing on to bradycardia before sinus rhythm was restored On January 7 1968 he had cardiac arrest but was easily resuscitated An ECG was not obtained as monitoring had been interrupted On the next day he had an attack of tachycardia which terminated in asystole during which he fainted Sinus rhythm recurred spontaneously Propranolol was started again in a dosage of 80 mg per day The attacks of tachycardia continued As they very often terminated in asystole causing fainting it was considered necessary to implant a pacemaker before the dosage of propranolol could be increased. So a transvenous demand pacemaker was inserted on January 1. and the dosage of propranolol subsequently increased to 1 0 mg per day This did not abolish tachycardia completely but the f

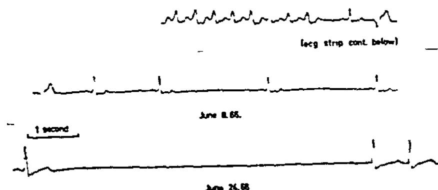


Fig Bipolar chest lead of case 1 Two upper strips (continuous, June 8 1966) tachycardia terminating in sinus bradycardia Lower strip (June 24 1966) sino-auricular block.



Fig 3 Standard leads I II and III of case 1 showing ECG after a bicycle ergometer test, July 1968 (200 kpm for 5 min 400 kpm for 5 min 600 kpm for 2 min.) Treatment propranolol 90 mg per day

quency of attacks was considerably reduced The pace maker effectively prevented fainting The patient was discharged on February 17 His chest X ray still showed an enlarged heart (760 ml/m) His blood pressure was 100/80 There were no signs of heart failure but he was extremely tired and severely incapacitated by intermittent claudication in his left leg He was seen in the out patient clinic on March 27 1968 when he reported two short lasting attacks of tachycardia. Otherwise his condi tion was unchanged He died suddenly in his home on April 12 1968 An autopsy was not performed

Case 3

Female born 1912 This woman has rheumatic mitral valvular disease for which a successful closed mitral valvulotomy was done in 1956 A slight diabetes is well controlled with an oral antidiabetic drug

She was first seen in this department in 1958 because of palpitations At that time her ECG showed a rather pronounced sinus arrhythmia and some atrial extrasys toles She was digitalized and remained well till 1963 when she started to have attacks of tachycardia For this reason she was readmitted in March 1964 ECG record ing during the attacks showed an atrial tachycardia This was not due to overdigitalization In between she had sino-auricular block causing dizzy spells and occasional fainting Because of a slight heart failure she was given a thiazide diuretic Antiarrhythmic treatment with a long acting quinidine preparation (Kinidin-duretter® 600 mg per day) was started and seemed to have some effect in the beginning However the attacks of tachycardia grad ually became more frequent and long lasting For this reason quinidine was stopped and in 1965 propranolol was given instead in dosa es up to 80 mg per day Dur ing this treatment the dizzy spells grew more frequent and she had several syncopes Addition of atropine 2 mg per day and reduction of the dose of propranolol did not change the situation. If treatment with propranolol was to be continued it was considered necessary to implant a pacemaker first in order to prevent fainting due to prolonged asystole However the patient was reluctant to receive this kind of treatment So propranolol was stopped in September 1967 The patient went on having dizzy spells fainting fits and frequent attacks of tachycardia

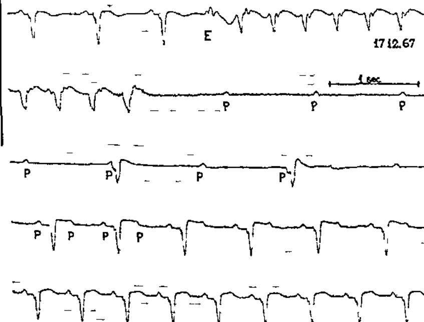


Fig 4 Bipolar chest lead of case 2, December 17 1967 1st strip initiation of tachy cardia by ectopic beat (E) nd strip 3 min 45 sec later abrupt change to entricular asystole which lasted for 14 sec 3rd strip complete a v block. 4th strip 2 1 a v block 5th str p sinus tachycard a 10 min after onset of the attack

Table II *Type of tachycardia and block in six patients complaining of palpitation and spells of dizziness or fainting*

Case no	Tachycardia Supra ventr	Ventr	Block Sino auric	Atrio ventr	Tachycardia terminating abruptly in block
1	−	+	+[s]	−	−
2	−	+	−	+[s]	+
3	+	−	+[s]	−	+
4	+	−	+	−	+
5	+	−	+[s]	−	+
6	+	+	+	−	+

s arrhythmia which caused syncope

implantation and propranolol Patient no 4 has had a demand pacemaker implanted but as a stable position of the catheter electrode has not been obtained antiarrhythmic drug treatment has not yet been started

DISCUSSION

To our knowledge patients of this kind presenting with complaints of both palpitation and dizziness or fainting and the electrocardiographic findings of tachycardia alternating with block have not been mentioned very often in the literature

Cohen et al (1) have reported the successful use of a pacemaker in a patient with supraventricular tachycardia alternating with sinus and nodal bradycardia They suggest that establishment of retrograde ventriculo atrial conduction prevented recurrence of the tachycardia In patient no 5 in this series in whom retrograde atrial activation also occurred during pacing of the ventricles a similar mechanism might have prevented atrial flutter thus offering a possible explanation why this arrhythmia recurred at the time of battery failure

A series of patients with attacks of supraventricular tachycardia and block in whom the combination of an antiarrhythmic drug and a pace maker was used with success has been reported to us by Sowton (5)

The conclusion of our experience with this small series is that patients complaining of both dizziness or fainting and palpitation should be investigated meticulously by long term ECG recording as their fits of fainting may be caused either by tachycardia or block or both Some of the patients might be relieved by means of drug treatment and others by implantation of a pacemaker whereas a combined treatment with antiarrhythmic drugs and pacemaker implantation may be necessary in several cases According to our experience it seems difficult to predict which kind of treatment will turn out most advantageous Therapeutical trials during continuous ECG supervision are warranted in such patients Before treatment with an anti arrhythmic drug is started we would advise the transvenous insertion of an interim catheter pace maker ready for immediate use in case asystole should be provoked by the antiarrhythmic drug as these patients are predisposed to develop heart block and asystole When permanent pacing is indicated it should be considered in each case which type of pacing—atrial or ventricular demand or fixed rate—should be preferred Atrial pacing which hemodynamically is superior to ventricular pacing (3) seems well indicated in patients without atrial tachycardia fibrillation or atrio ventricular block Demand pacing is suitable when the purpose is to prevent syncope caused by bradycardia or block whereas fixed rate pacing might be preferable when the aim is suppression of tachycardias (2 4)

Table III *Kind of treatment in six patients with alternation of tachycardia and block*

Treatment	No of pats	Cases no
Pacemaker only	3	(4)[a] 5 6
Antiarrhythmic drug + pacemaker	3	1 2 3

[a] For reasons given in this patient s case history he is not yet on treatment with an antiarrhythmic drug

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HEREDITARY HEPATIC PORPHYRIAS

Gene Penetration Drug Sensitivity and Subdivision in the Light of Systematic Family Studies

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Abstract On the basis of systematic family investigations of Danish porphyria patients through ten years with recording in a card index, the gene penetration has been studied in different families. The suggestion of Waldenstrom that gene penetration might vary was confirmed as some families had many manifest cases and a high number of latent cases excreting high concentrations of metabolites while others had only one manifest case and few latent excretors with low metabolite concentration. Further an intermediate status of different degrees exists as illustrated by Figs 1-6 It is concluded that the large families with acute porphyria in Northern Sweden and with variegate porphyria in South Africa constitute extremes the most frequent finding in other countries being families with considerably less pronounced gene penetration Observations further suggest that the sensitivity to drugs—barbiturates etc—as provocators of porphyric attacks varies with the gene penetration prevailing in the family thus the risk of drug provoked attacks seems to be much higher in Sweden and South Africa than in Denmark. It is therefore an important point in the prophylaxis of the porphyric attack to study the family of every patient to get an impression of the gene penetration and drug sensitivity prevailing in that particular porphyric family It is recommended that a complete porphyrinological investigation be performed in all porphyric patients as well as their close relatives—parents siblings children—i.e screening tests for porphyrin in urine and faeces supplemented by quantitative analyses if positive and—last but not least—ion exchange chomatographic analysis for both porphobilinogen (PBG) and delta aminolaevulinic acid (ALA)

This should be done not only in acute intermittent porphyria (AIP) and variegate porphyria (VP) but also in hereditary coproporphyria (HCP) and hereditary porphyria cutanea tarda (PCT H) Examples from the Danish material are given The findings indicate that the subdivisions between different forms of hereditary hepatic porphyrias are more or less artificial as really every family constitutes its own form of porphyria Even a rare form such as HCP varies profoundly from one family to another The diagnostic values of systematic family investigations is exemplified and stressed

While porphyrins are well defined compounds the concept of porphyria is far more floating Actually every investigator actively engaged in porphyria research has his own version of the definition and subdivision of this group of diseases for that of the writer the reader is referred to his recent survey on the clinical chemistry of porphyrias (27) It therefore seems evident that the subdivision and nomenclature of porphyrias can only be provisional and tentative and do not constitute a firmly established system

During his study of porphyrias in Denmark for over 20 years a study which has included systematic family investigations with chemical tests for ten years the writer (23-25) became increasingly intrigued by the difference between his findings in Denmark and those of Waldenstrom and his group in Sweden (18 19 22) Simultaneously the Waldenstrom group became similarly intrigued by the differences between their own findings and those of the South African workers concerning variegate porphyria (7)

A way of explaining these unquestionably real differences between the observations on different forms of hereditary hepatic porphyrias in different countries was pointed out by Waldenstrom and Haeger Aronsen (19) who suggested that the gene penetration in the big family with acute intermittent porphyria (AIP) in Northern Sweden was exceptionally high It seemed natural to go further and suggest that the gene penetration in the large South African family with variegate porphyria (VP) was correspondingly high as this would explain the differences observed between VP in South Africa and in other countries Further

observations suggest that drug sensitivity may be related to gene penetration because the Swedish and South African experiences of the risk of latent porphyrics taking barbiturates contrast clearly with Danish observations Finally our own studies of a family in central Jutland with hereditary coproporphyria (11) suggest that this disease cannot really be an entity but must consist of a group of diseases with varying clinical and chemical features and severity

The author therefore believes that every porphyric family has its own characteristic disease which has once origined as a mutation perhaps in one locus characteristic of the hepatic porphyrias perhaps in different loci close to each other Consequently every family should constitute a special entity as regards symptoms gene penetration and sensitivity to pregnancy and drugs—and the evidence from the writer s record of Danish porphyria families was found to give some support to this view This hypothesis does not contradict Watson s (20) concept of hepatic porphyria as a melting pot our concept of a series of families with different diseases being based on our systematic family studies which are possible in Denmark while similar studies can hardly be successfully perform d in most other countries

METHODS

The analytical methods employed have been described by With (3 27) The morning urine of every patient and relative was subjected to ion exchange chromatographic determination of porphobilinogen (PBG) and delta aminolaevulinic acid (ALA) as well as to screening of porphyrins the faeces were screened for porphyrins Quantitative analysis was performed if screening was positive The patients from the last two years were further studied with thin layer chromatography of extracts of their excreta by the methods introduced by the writer employing talc layers (26 28)

Family investigation was offered to every patient. This proceeded as follows First the patient or one of the patient s relatives constructed a table of the close relatives (parents, siblings children) with names and addresses Then mimeographed standard letters were sent from our laboratory to the relatives, accompanied by material for collection of urine and faeces The letters contained a short account of the porphyria disease in their relative and the risk involved as well as the advantage of knowing beforehand one s eventual state as porphyria carrier It was made clear that all analyses are carried out without cost to the relatives as a scientific service On average about 50 of the relatives responded by sending the materials requested A substantial number of the relatives not responding to the letters were as a rule contacted later through cooperative members of the family When the analytical data were ready both the relatives and their physicians received standard letters explaining the implication of being a carrier should this prove to be the result of the analysis, or explaining that the analyses exhibited normal results on this occasion, which does not with certainty exclude carrier status The letters also mentioned the drugs which have to be avoided and further stressed the importance of informing all physicians and hospitals with whom the relative comes into contact about their certain or possible state as a porphyria carrier

In the long run as a rule 30–70 % of the relatives were subjected to study in this way but in some families the coverage approached total, because we had the opportunity to contact the subjects several times when new episodes of porphyria developed in their families. The continued efforts through the last ten years have thus resulted in a rather good coverage of several Danish porphyric families but the material is still far from complete although the interest of Danish hospitals and physicians in our work is increasing

RESULTS

Our findings are illustrated by typical examples from the Danish porphyria register in Figs 1–6 Further examples will be found in With (23) Thiele and With (16) as well as for hereditary coproporphyria (HCP) in Lomholt and With (11) In Figs 7–9 examples of non porphyric families studied by the same technique are presented as controls Further our laboratory possesses a large series of analyses for PBG and ALA in urine as well as porphyrin screening in urine and faeces by the methods mentioned above according to which below 1 % of normal adult subjects have ALA above 0 60 and PBG above 0 30 mg per 100 ml of urine or positive screening tests in urine or faeces for porphyrins This material has been partially published (27) but is continually expanding and at the moment comprises more than a thousand subjects

In the figures the letter A designates the first generation B the second etc Numbers following the letters refer to the sequence in the sibships Thus A 7 is number seven in the first generation B 2 3 is number 3 among the offspring of A 2 C 5 1 3 is the third child of B 5 1 etc The sex of the subjects is indicated by symbols under the numbers If the sex symbol is placed within brackets it means that the excreta of the subject in question have not been subjected to chemical study If the sex symbol is without brackets and underlining the excreta have been studied and found normal if the sex symbol is underlined

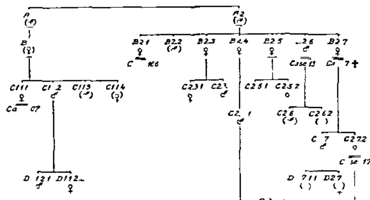


Fig 1 Family (4 generations) with 27 members, 5 cases of porphyria one (case 7) died during a porphyric attack. A 1 A 2 and B 11 are registered as carriers because of cases of porphyria among their descendants Seventeen family members were studied. 5 patients, one carrier (B 2.5) and 11 with normal ex cretion. B 2.5 had PBG 5 0 ALA 0 67 mg/100 ml and normal faeces C 2 4 1 and C 2 7.2 (case 117) are married but without offspring yet.

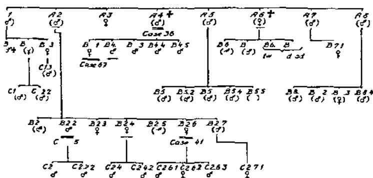


Fig 2 Family (3 generations) comprising 48 members 4 cases of AIP and 6 carriers 17 sujects were studied Case 36 died from porphyria before the family investigation and, together with his daughter (case 69) was reported on by Budolfsen et al (Acta psychiat. scand Suppl 108 71 1956) Cases 51 and 141 are both extremely severe and protracted, the former having extensive residual pareses 11 years after the first attack. All the subjects studied had normal faecal porphyrin. The figures for PBG and ALA (mg 100 ml) were as follows B 2.4 PBG normal ALA 0 80 B 4.2 1 40 2 00 C 2 4.2 normal 0 70 C 2 6 2 3 1 0.3_ A_ and A 4 were registered as carriers because of the cases of AIP among their offspring

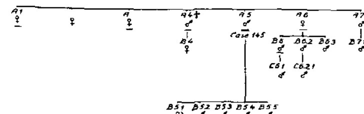


Fig 3 Family (3 generations) comprising 19 members, of whom 17 were studied one patient and 7 carriers The faeces of A 5 (case 145) showed coproporphyrin 340 and protoporphyrin 135 μg per g dry matter during the attack. The faeces were normal in all the relatives studied except A 1 who showed coproporphyrin 13? and protoporphyrin 37 μg per g dry matter The PBG and ALA values were A 1 PBG 2. 0 ALA 1 30 A 3 0 72, 0 65 A 4 3 10 1_0 A 5 (case 145) 2 60 1.50 A 6 1.25 0 6 B 6 1 11 ? 4 30 A 4 later developed a vascular cerebral affection with pareses at that time PBG was 2 60–6 40 and ALA 1 80–4.50 If he had not been known as a latent porphyric an erroneous diagnosis of porphyria as cause of his pareses might have been made This family shows features of both AIP and VP ("mixed porphyria") It is remarkable that the carrier with the highest urinary excretion had normal faecal porphyrin.

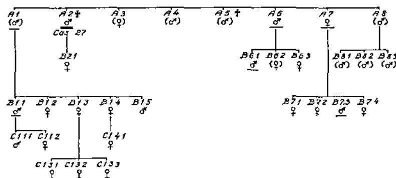


Fig 4 Family (3 generations) with 30 members 20 were studied and only 3 showed increased excretion and only of ALA The figures were B 1 1 0 70 B 6 1 0 62 and B 7 3 0 62 mg/100 ml As our normal material comprising more than 1000 cases exhibits ALA above 0 60/100 ml in about 1 of the cases this finding strongly suggests porphyria The patient (case 27) was a Danish physician working in a remote part of the Faroe Islands who died from a protracted attack diagnosed as AIP Chemical verification was not possible The daughter of the patient (B 2 1) was suspected of AIP but showed normal excretion Faecal porphyrins were normal in all the subjects The family is regarded as one of AIP with low gene penetration

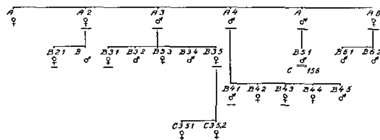


Fig 5 Family (3 generations) with 23 members who were all studied one patient (B 5 1 case 156) and 13 carriers A ? A 3 and A 4 had normal excretion and were registered carriers for genetical reasons Faecal porphyrins were normal throughout The PBG and ALA figures were A 5 PBG 0 39 ALA 0.26 A 6 0 37 0 24 B 2 1 0 30 0 83 B 3 1 0 38 0 39 B 3.2 0 20 0 69 B 3 5 1 00 0 46 B 4 1 0.22 0 63 B 4 3 0 47 0 61 C 3 5 1 0 31 0 79 C 3 5 2 0 47 0 61 mg per 100 ml urine These values are low but nevertheless definitely abnormal The patient suffered from polyradiculitis beginning with abdominal pain and later complicated with respiratory and cardiac arrest from which he recovered His excreta were studied and found normal twice during the later course when he was still paretic PBG and ALA were not measured during the acute stage but a slight elevation of porphyrins was found in his urine at that time This case from Copenhagen City Hospital Surgical Department I was studied in collaboration with S A Damgaard Nielsen M D

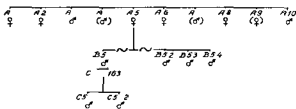


Fig 6 Family with 17 members 14 were studied and all found to be normal except the patient (B 5 1 case 163) an illegitimate child and his two sons The patient s father was not available for study The patient was admitted to the Neurological Department, County Hospital Holstebro Jutland (Head P Berggren M D) for polyradiculitis and showed normal PBG and slightly increased ALA during the paralytic stage (PBG 0 30 and ALA 0 70 mg) and normal faecal porphyrins His sons both showed normal faecal porphyrins and normal PBG but elevated ALA C 5 1 1 0 76 and C 5 1 ? 1 10 mg per 100 ml

once the excreta have been found to contain definitely abnormal quantities of porphyrins and/or precursors on at least one occasion or the subject in question has to be regarded as a carrier because of proved carriers among the offspring if the sex symbol is doubly underlined it means that the subject has had at least one episode of clinical manifest porphyria either abdominal neuropsychic or cutaneous

Individual data are found in the explanations to the figures

DISCUSSION

As seen from Figs 1–6 and the figures of With (23) some porphyric families have several manifest cases—each with its own number in the Dan

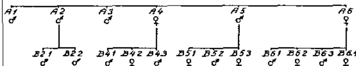


Fig 7 Normal family exhibiting negative values throughout in study of 18 members (two generations) All living family members were studied The study was performed because A 2 was suspected of AIP (recurrent attacks of abdominal pain)

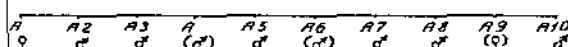


Fig 8 Family consisting of 10 siblings of whom 7 were subjected to study because one (A 2) was suspected of AIP (recurrent attacks of abdominal pain) All exhibited negative urine and faecal screening for porphyrins PBG below 0 30 and ALA below 0 60 mg per 100 ml

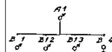


Fig 9 Study of the four descendants of a patient with lead intoxication (A 1) The patient showed ALA 0 76 and 0 67 mg per 100 ml on two occasions All the five subjects showed negative urine and faecal screening for porphyrins and PBG below 0 30 mg per 100 ml The four children all had ALA below 0 60 mg per 100 ml

ish porphyria register—while the latent carriers are not numbered In these families there is a tendency to relatively high concentrations of PBG and ALA among the carriers but the excretion is not constant and rather often PBG is normal with definitely increased ALA thus confirming our earlier results (23) and being contrary to the experience of the Swedish group Waldenstrom and Haeger Aronsen (18 19) found that in their extensive material of acute intermittent porphyria mostly consisting of members of the huge porphyric family in Northern Sweden proved carriers with normal PBG excretion are very rare which is also the case with carriers with increased ALA and normal PBG The same findings are recorded by Wetterberg (22) who studied porphyria in a psychiatric material from Sweden These results cannot be due to differences in technique because the modification of the Mauzerall Granick method used in our Danish series must if anything give higher PBG values than the original method used by the Swedish investigators The only explanation of this discrepancy therefore seems to be differences in the genetic outfit between carriers of AIP in Denmark and Sweden This possibility had been mentioned by Waldenstrom and Haeger Aronsen (19) who suggested that the gene penetration in the big Swedish AIP family might be exceptionally high We here extend this hypothesis further by assuming a variation in gene penetration from family to family as exemplified in our Figures Figs 1 and 2 being examples of relatively high gene penetration although considerably lower than found in the big Swedish AIP family and Figs 3–6 exemplifying families with lower gene penetration Our hypothesis includes the concept that negative PBG but positive ALA as well as intermittent excretion and relatively low concentrations in carriers indicates a low gene penetration while the opposite findings indicate a high one Further we came to the conclusion that the risk for the carriers of developing porphyric attacks because of drug administration—especially barbiturates—most likely varies in parallel with the gene penetration It was from Sweden that the risk of barbiturates was originally pointed out but in Denmark the carrier state is so widely distributed and barbiturate consumption so common that many porphyrics must have taken barbiturates several times and as porphyric attacks are rare barbiturate sensitivity cannot be high Add to this the porphyric epileptics who have taken large doses of barbiturate for long times (9 10) Finally the first case of a porphyric attack published in Denmark (12) received 500 mg of diemal twice and later 100 mg of phenemal as daily injection for 16 days during his illness His porphyria did not get worse through this treatment but recovery began immediately after it was stopped This is not mentioned in the publication but was only recently found out during the author s study of the original case record (from A W S Sørensen M D Alborg County Hospital Alborg) for at the time of the publication the role of barbiturates in producing porphyric attacks was unknown According to Swedish and South African experience this patient should be long dead but he developed only a moderately severe attack with

limited pareses of the arms abdominal pains and mental confusion Also in another of the early Danish cases—no 26 from Svendborg County Hospital—where the writer recently had the opportunity of studing the original hospital records recovery from an attack of acute porphyria took place in spite of continued administration of 90 mg of phenemal daily and 100 mg of allypropymal as hypnotic in the evening for several weeks during the attack

To verify our hypothesis extended studies with family investigations like the ones reported here will be necessary and we therefore recommend such analyses as a routine in all cases of porphyria when circumstances allow having regard to the fact that such investigations require much time and skill We wish to emphasize however that if properly administered much of the work can be done by secretaries and technicians making the burden on the physicians considerably less than it appears on first consideration

Further we wish to point out the diagnostic value of family investigations in many cases of paralytic porphyria in which the excretion of metabolites not infrequently becomes normal long before the pareses disappear We have several examples of this including our case no 156 Fig in which excretion was completely normal when finally studied by adequate methods and the family had several members with slightly increased excretion of metabolites and one with moderately increased Another examples is case no 163 Fig 6 in which only ALA was increased when the urine was subjected to adequate study in the paralytic stage In this connection it is worth remembering that in South African VP the excretion of PBG and ALA during acute attacks usually becomes normal within a few weeks after the beginning of the attack normal excretion of PBG and ALA being frequently seen while pareses are still present (5) Thus some of our cases with AIP and low gene penetration are similar to South Africa s VP in this respect

Family investigations may also be valuable for avoiding an erroneous diagnosis of porphyria It is now recognized that porphyria may coincide with other diseases which clinically may mimic a porphyric attack—as for example inflammatory spinal lesions (21) polyarteritis nodosa (Becker (1) and Waldenstrom (17)) and disseminated lupus erythematosus (8) and if such diseases occur in an unknown latent porphyric constantly excreting high concentrations of metabolites this can easily lead to a false diagonsis of porphyria But if the porphyric is known beforehand from a former family investigation this error becomes less likely

Our hypothesis is also supported by the differences between observations on variegate porphyria in South Africa and Sweden as recently expressed by Hamnstrom et al (7) The South Africans (2 3 4 6) find that increased faecal porphyrin excretion is practically constant in VP carriers while the Swedish investigators could not confirm this and found a carrier in their family B who was normal in all the chemical tests as well as one with normal PBG and increased ALA in their family C

Danish experience of VP is limited as our material consists of only four VP patients of whom two are adopted children Three of these cases were studied by the writer and in two faecal porphyrin excretion was followed through several years and found to be intermittent. In one a family investigation was performed All members of two generations a total of nine subjects were studied and only the patient showed elevated faecal porphyrins Two of the others showed slightly increased ALA the remainder was normal These observations suggest definite differences between VP in Denmark and South Africa Watson (20) too emphasized the differences in VP as described by South African workers and the mixed porphyria cases from his United States material which resembled them One of the Danish cases of VP (case no 118) is of special interest (Chief physicians O Remvig Fysiurgisk Hospital Hornbæk and H Brodthagen Finseninstitutet Skin Department placed this patient at our disposal)

Woman, born 1961 adopted child developed a severe attack of paralytic porphyria in October 1964 She was of dark hue but had no hyperthricoses. During the beginning of the attack her urine PBG was 2 15 and ALA 1 30 mg/100 ml and her faecal porphyrin 1700 μg g dry matter consisting of coproporphyrin without demonstrable dicarboxylic porphyrins Typical crystalline coproporphyrin III ester was prepared from one week s collections of her faeces in Nov 1964 and May 1965 c 50 mg on each occasion. She recovered in the course of one year being discharged from hospital with only partial paresis of the left thumb remaining in Nov 1965 During the first year of her disease PBG and ALA had been elevated and on discharge PBG was still ? 2 mg per 100 ml There had b en a few episodes of abdominal pain, but no fresh

during the phase of recovery from Nov 1964 to ov 1965
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A complete porphyrinological investigation thus comprises screening of urine and faeces for porphyrins and quantitative analysis if positive tests are found as well as ion exchange chromato graphic determination of both PBG and ALA Further it is recommended to include thin layer chromatographic study of the porphyrins by the talc method which is easy rapid and ch ap (26 28) If the porphyrins are extracted from faeces or urine with a mixture of equal volumes of amyl alcohol glacial acetic acid and ether and from this extract with 1 *M* HCl which is then applied to chromatographic talc plates beautiful chroma tograms can be obtained in 10–15 min in which also 7 carboxyl porphyrin diagnostic of PCT can be clearly separated from uroporphyrin (29)

ACKNOWLEDGEMENT

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limited pareses of the arms abdominal pains and mental confusion Also in another of the early Danish cases—no 26 from Svendborg County Hospital—where the writer recently had the opportunity of studing the original hospital records recovery from an attack of acute porphyria took place in spite of continued administration of 90 mg of phenemal daily and 100 mg of allypropymal as hypnotic in the evening for several weeks during the attack

To verify our hypothesis extended studies with family investigations like the ones reported here will be necessary and we therefore recommend such analyses as a routine in all cases of porphyria when circumstances allow having regard to the fact that such investigations require much time and skill We wish to emphasize however that if properly administered much of the work can be done by secretaries and technicians making the burden on the physicians considerably less than it appears on first consideration

Further we wish to point out the diagnostic value of family investigations in many cases of paralytic porphyria in which the excretion of metabolites not infrequently becomes normal long before the pareses disappear We have several examples of this including our case no 156 Fig 5 in which excretion was completely normal when finally studied by adequate methods and the family had several members with slightly increased excretion of metabolites and one with moderately increased Another examples is case no 163 Fig 6 in which only ALA was increased when the urine was subjected to adequate study in the paralytic stage In this connection it is worth remembering that in South African VP the excretion of PBG and ALA during acute attacks usually becomes normal within a few weeks after the beginning of the attack normal excretion of PBG and ALA being frequently seen while pareses are still present (5) Thus some of our cases with AIP and low gene penetration are similar to South Africa s VP in this respect

Family investigations may also be valuable for avoiding an erroneous diagnosis of porphyria It is now recognized that porphyria may coincide with oth r diseases which clinically may mimic a porphyric attack—as for example inflammatory spinal lesions (21) polyarteritis nodosa (Becker (1) and Waldenstrom (17)) and disseminated lupus erythematosus (8) and if such diseases occur in an unknown latent porphyric constantly excreting high concentrations of metabolites this can easily lead to a false diagonsis of porphyria But if the porphyric is known beforehand from a former family investigation this error becomes less likely

Our hypothesis is also supported by the differences between observations on variegate porphyria in South Africa and Sweden as recently expressed by Hamnstrom et al (7) The South Africans (2 3 4 6) find that increased faecal porphyrin excretion is practically constant in VP carriers while the Swedish investigators could not confirm this and found a carrier in their family B who was normal in all the chemical tests as well as one with normal PBG and increased ALA in their family C

Danish experience of VP is limited as our material consists of only four VP patients of whom two are adopted children Three of these cases were studied by the writer and in two faecal porphyrin excretion was followed through several years and found to be intermittent In one a family investigation was performed All members of two generations a total of nine subjects were studied and only the patient showed elevated faecal porphyrins Two of the others showed slightly increased ALA the remainder was normal These observations suggest definite differences between VP in Denmark and South Africa Watson (20) too emphasized the differences in VP as described by South African workers and the mixed porphyria cases from his United States material which resembled them One of the Danish cases of VP (case no 118) is of special interest (Chief physicians O Remvig Fysiurgisk Hospital Hornbæk and H Brodthagen Finseninstitutet Skin Department placed this patient at our disposal)

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INCREASED ENTERIC FORMATION OF HISTAMINE IN CHRONIC PANCREATITIS

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Abstract Patients with chronic pancreatitis in contrast to patients with acute pancreatitis were found to have increased excretion of conjugated histamine in urine (7 of 8 patients) A close correlation existed between the amounts of conjugated histamine in urine and the concentration of histamine like activity in the feces Fecal samples from patients in contrast to samples from controls, possessed a capacity to form histamine like activity from L histidine and N acetyl L histidine Sterile filtrates on the other hand possessed no such ability Feces from patients had a significantly lower pH than control feces This may be a predisposing factor for the observed histamine formation since the bacterial L histidine dicarboxylase has a low pH optimum

It is concluded that the excess conjugated histamine in urine in these patients originates in the lumen of the gastrointestinal tract

Various histamine metabolites seem to be present in human urine 1 4 methylhistamine (8) mono- di and trimethylhistamine (methyl groups in the side chain of histamine (12) 1 4 methylimidazole acetic acid (1 5 methylimidazole acetic acid (10 28)) *N* acetylhistamine (29) and also probably 4 (5)-imidazolyl-ethane 2-ol (18) Injected labelled histamine is also converted to imidazole acetic acid and imidazole acetic acid riboside and excreted as such in the urine (20)

N acetylhistamine is apparently a rather unimportant urinary metabolite of parenterally administered labelled histamine (*N* acetylhistamine/histamine ratio in urine 1 – < 0 2 (13 20)) The so-called conjugated histamine in the urine which signifies the increment in biologically active histamine upon acid hydrolysis appears spontaneously in the urine in a relatively higher proportion (mean conjugated histamine/free histamine ratio in healthy individuals 2 4 (23)) Nevertheless the urinary conjugated histamine seems to consist wholly (or partly?) of *N* acetylhistamine The explanation of this apparent inconsistency may be that the conjugated histamine in urine (and *N* acetylhistamine) has a dual origin both endogenous and exogenous (6)

There seem to be at least three different types of increased urinary excretion of conjugated histamine in man

1 Increased excretion secondary to ingestion of histamine (1)
2 Increased excretion secondary to ingestion of *N* acetylhistamine (25)
3 Increased excretion of conjugated histamine in the urine as encountered in some patients with myotonic dystrophy (26)

In myotonic dystrophy there is a close correlation between the urinary excretion of conjugated histamine and the fecal excretion of histamine like activity (26) The primary abnormality in this chain of events appears to be increased formation of histamine like activity in the intestinal lumen The formed histamine is conjugated and absorbed or vice versa and then excreted in the urine in the conjugated form These findings add weight to the view (4) that there is an intestinal dysfunction as well in myotonic dystrophy

It may be anticipated that some disorders *primarily* affecting the gastrointestinal tract could have the same abnormality of histamine metabolism Therefore in order to obtain information regarding the specificity of increased urinary excretion of conjugated histamine in myotonic dystrophy a study of the urinary excretion of conjugated histamine in gastrointestinal disorders was carried out (27)

Table I *Urinary and fecal excretion of histamine in patients with chronic pancreatitis*

Case no	No of obs urine	Urinary hist µg/24 h Free Mean	Range	Conjugated Mean	Range	HA[a] Mean (range)	Fecal pH Mean (range)	Commentary
1	8	40	23–90	2850	920–7100	88 (47–140)	6 2 (6 0–6 6)	
2	2	16	8–23	1250	600–1900	—	—	
3	5	75	11–160	510	340–1000	24 (13–38)	6 2	
4	4	3	< 2 4–6	,00	87–350	3 7 (0 5–6 9)	6 3 (5 6–7 0)	Gastric resection 17 y previously
5	1	34	34	200	200	—	—	
6	3	20	< 6–30	140	38–220	0 4	6 1	
7	2	27	6–47	100	55–150	—	—	
8	2	8	4–11	36	25–47	0 0.	5 4	*Pulmonary symptoms* Sweat test *normal*
9	3	14	12–17	410	350–480	0 14 (0 05–0 23)	7 0 (6 9–7 2)	No steatorrhea at time of study Gastric ulcer Laparotomy Cystic pancreas Chr inflammation
10	1	4	4	74	74	—	—	No steatorrhea at time of study Diagnosis only probable
11	2	14	5–[illegible]	155	140–170	1 8	6 9 (6 8–6 9)	Diagnosis only probable
12	1	7	7	43	43	—	—	Diagnosis only probable
Control series (23)		12 6	2–31	30 0	1–99	< 0 17		

[a] Histamine like activity in the feces in µg base/g wet weight

Contrary to expectation normal or only sporadically elevated excretion was found in all disorders examined except one in patients with steatorrhea due to chronic pancreatitis (a total of 4 patients) there was significantly increased excretion of conjugated histamine

The present communication summarizes studies intended to substantiate this finding and to elucidate the mechanism producing the increased output of conjugated histamine in urine in chronic pancreatitis

MATERIAL

Eight cases considered to suffer from chronic pancreatitis with steatorrhea were studied Patients nos 9 and 10 (Table I) did not have steatorrhea at the time of study Due to the paucity of cases three cases with only probable chronic pancreatitis were included (viz. nos 10–12

As is evident from Table VII the patients with acute pancreatitis were heavily medicated and this might have influenced the results of the histamine studies In particular antihistaminics cortisone and meperidine are pertinent in this connection antihistaminics influence the biological assay of histamine cortisone increases the excretion of histamine in the urine (17) and meperidine is a releaser of histamine (31)

Some of the patients with acute pancreatitis were in poor condition particularly no 16 but all survived the attack Patient no 15 died from mesenteric vein thrombosis approximately three months later

No restrictions were given as to diet during study Most patients with chronic pancreatitis ingested a low fat diet. Most patients with chronic pancreatitis also used Combizym®

METHODS

Free and conjugated histamine in urine

With some minor modifications Dunér and Pernow's method (5) has been employed Urine was collected in 24 hour portions, but the collections were not carried out in a metabolic ward and urinary creatinine determinations were not made to check the completeness of collection *Culture and microscopical examination of urine* were carried out in some patients and there were no signs of infection

Free and conjugated histamine in gastric and duodenal juice

These parameters were measured as described elsewhere (26) using samples obtained during secretin tests prior to injection of secretin

Histamine like activity in the feces

For the estimation of the free fraction a method described in detail elsewhere (2?) has been employed This

method is inaccurate for estimating the conjugated fraction in the presence of high levels of histamine like activity In order to identify or quantify the conjugated fraction in stool samples three methods were used

1 Paper chromatography (see later) for qualitative purposes
2 The free and conjugated fractions were separated on an Amberlite IRC-50 column as described elsewhere (method 2 26) and subsequently estimated semiquantitatively
3 The histamine like activity in the fecal sample concerned was estimated Another aliquot of the homogenized fecal sample was incubated with diamine oxidase in order to inactivate the histamine like activity partially or completely (this was checked by a biological technique) after which the regular technique (22) for quantifying conjugated histamine was adopted

Fecal incubations

Incubations were carried out in the following manner The whole fresh specimen was homogenized and an aliquot approximately 5 g was mixed carefully with 15 ml distilled water Incubations were carried out aerobically without stirring at 37 C for 20–24 h with 1) 300 mg of L histidine monohydrochloride monohydrate or 2) 300 mg of *N* acetyl L histidine 3) C histamine dihydrochloride 3–100 μg (specific activity 116 μC/mg) or ^{14}C L histidine 2–60 μg (specific activity 35 mC/mmole) Biological assay and autoradiography showed that the batches of both conventional and labelled L histidine (30) and *N* acetyl L histidine contained only negligible quantities of histamine In other experiments 1–3 g of patients feces and 1–15 g of control feces were mixed homogenized and incubated in the same way

The pH in the incubation mixtures was not adjusted after addition of the chemical substances

After incubation the mixture was centrifuged and the supernatant fluid filtered by suction in a Buchner funnel The pH was adjusted to pH 7 4 before direct assay on the guinea pig ileum. A few samples were subjected to the aforementioned extraction procedure (22) used for histamine like activity in the feces

All values for histamine refer to the base and almost invariably represent the mean of duplicate analyses Although recovery studies have been carried out systematically no corrections have been made for losses during the procedures

Fecal pH in freshly expelled whole sample homogenates (weight of feces in g × 3 = ml of distilled water) was measured potentiometrically with a glass electrode (Radiometer Copenhagen)

Indican excretion in urine was measured as outlined by Meiklejohn and Cohen (15) Urine was collected with 60 ml 1 2 *N* hydrochloric acid instead of toluene as recommended by the authors

Paper chromatography

The following systems were used for two-dimensional descending paper chromatography of fecal extracts (Whatman s paper no 1) system 1) *n*-butanol/acetic acid distilled water (4 1 1 v/v) and system 2) *n* butanol saturated with 20 NH OH As histamine and 1 4 methylhistamine are poorly separated in this chromatographic system, another moving phase was used to separate them 3) phenol/chloroform/ethanol/ammonia (14 14 10 2 v/v (T White personal communication)) It was not necessary to carry out desalting of the fecal extracts since the moving rates of internal standards were not notably altered

Fecal extracts were prepared as described under fecal incubations Generally 50 μl of the filtrates were spotted on the chromatograms The chromatograms were stained with either diazotized *p*-nitroaniline or diazotized sulphanilic acid

Kodak X ray films were exposed to the radioactive chromatograms for 4–6 weeks No quantification of the radioactive metabolites was attempted in these experiments

L histidine loading orally

After a control day 10 g L histidine monohydrochloride monohydrate was ingested with 200 ml tap water at 8 a.m after overnight fasting On two occasions the oral dose was ingested also on the subsequent morning The patients ate their regular meals during the study

Oral sulfa treatment

Phthalylsulfathiazole (AFI Fftalyl®) was administered with the following doses 7 g initially then 10 g per day for 5 days No stool cultures were taken to check the effect of the treatment

RESULTS

Patients with Chronic Pancreatitis

Histamine in urine

The findings are condensed in Table I Seven of eight patients whose diagnosis was considered rather definite had an augmented output of *conjugated histamine* (88 %)

One of three patients with only probable diagnosis also had this anomaly In none of the patients was the mean excretion lower than the mean control excretion The difference between patients and controls was statistically significant both for the first eight cases and for the whole series ($P = 0\ 05$)

The *free fraction* was elevated in three cases with calculations based on unweighted values and in four cases when based on weighted values All cases with augmented output of free histamine also showed augmented output of conjugated histamine The mean excretion of free histamine did not differ significantly from the normal one ($P > 0\ 1$) Not only was the excretion of conjugated histamine more frequently elevated than that of free histamine but also quantitatively more

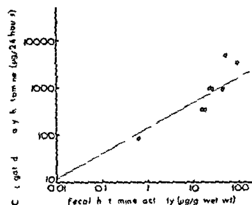


Fig 1 Logarithmic values for conjugated histamine in urine plotted against logarithmic values for histamine like activity in the feces Altogether 17 corresponding samples of feces and urine from 7 patients with chronic pancreatitis Correlation coefficient $r = +0\ 88$ $(P < 0\ 001)$ Equation of regression log conj histamine urine = 0 55 log histamine feces + 2 15

heavily affected The conjugated/free histamine ratio for the whole present series was 22 8 against a control value of 2 4 (23)

The statistical calculation both for free and conjugated histamine were based on unweighted values These values were more representative of the material since more samples were obtained from patients with high than from patients with low urinary excretion of the respective substances

Histamine like activity in the feces

Seventeen samples were obtained from seven patients With one exception increased values were found In every instance of increased excretion of conjugated histamine in the urine elevated histamine like activity was present in the corresponding fecal sample (Table II)

The figures for urinary conjugated histamine and fecal histamine like activity in corresponding samples were recalculated to a logarithmic scale and plotted against each other The correlation between the urinary and fecal parameters was close (Fig 1)

The contractions of the guinea pig ileum caused by the bioactive substance in feces mimicked those caused by authentic histamine diphosphate in every respect An antihistaminic agent diphenhydramine hydrochloride was occasionally added to the bath during assay The fact that it antagonized the contractions caused by the eluate and those caused by authentic histamine to the same degree (19) was taken to indicate that the substance tested was biologically identical with histamine Addition of atropine or dihydroergotamine to the bath was without any influence Nevertheless the biological activity was designated histamine like activity since it was more stable on incubation than added histamine and since clearcut results as to the identity of the substance were not obtained by chromatography (see later)

With special methods for separation of histamine and conjugated histamine (see Methods) it was found that a conjugated fraction was present in the feces as well (Table II)

Table II *Simultaneous estimation of histamine like activity and conjugated histamine in feces from patients with chronic pancreatitis*

Case no	Method no [a]	Histamine in feces (µg/g wet weight)	
		Histamine	Conjugated histamine
1	2	20	0 5
[illegible]	2	91	5 8
1	2	117	14
3	2	9 2	?8
3	2	8 3	32
3	3	2?	8 3
5	3	0 44	0 14

[a] See Methods (histamine like activity in the feces)

Table III *Histamine in gastric and duodenal juice in patients with pancreatic disorders*

Case no	Diagnosis	Histamine (µg base/ml)	
		Gastric	Duodenal
1	Chronic pancreatitis	<0 07/<0 06[a]	<0 07/<0 4
13	Chronic pancreatitis	—/ 0 2	—
14	Insulinoma	—	0 0
	Control values	<0 03/<0 018	<0 6/<0 1

[a] Free histamine/conjugated histamine

Histamine in gastric and duodenal juice

Normal or only slightly elevated values were found (Table III) the values being of the same order of magnitude as in myotonic dystrophy (26)

ecal incubations

shown in Table IV huge quantities of hista were formed when feces from patients were bated with histidine In these experiments the H was lowered because of the addition of the ino acid In control experiments however with pH adjusted to 6 5–7 0 an increased forma ı of histamine also took place Table IV demon that patients feces in contrast to control s could form histamine like activity from *N* ,l L histidine In a few experiments sulfa and histidine were added to patients feces This ˡ in a low histamine formation even at an pH Sterile filtrates (Millipore Filter pore iameter 0 22 μ) of feces possessed no ability to form histamine Patients feces seemed to be able to dispose of *N* acetylhistamine and in the course of this process a free fraction evolved as in con trol feces

Inherent fecal histamine like activity proved remarkably stable on incubation under the regular experimental conditions even when the incuba tion period was prolonged to five days Inherent histamine like activity on incubation was reduced at a much slower rate than authentic histamine by diamine oxidase

From Table V it appears that with one excep tion addition of control feces to feces with high inherent histamine like activity led to a marked reduction of the activity

Table IV *Formation of histamine like activity (HA) in the feces (5 g samples) (chronic pancreatitis) after addition of* L *histidine (222 mg of the free amino acid) or N-acetyl* L *histidine (237 mg of the free amino acid)*

Case no	Control HA	Additional activity after addition of L histidine	*N* acetyl L histidine
1	47	26 000	600
1	—	55 000	1460
1	86	95 000	—
1	—	49 000	—
1	—	140 000	—
1	—	40 000	—
1	55	106 000[b]	1420
3	13	70 000	—
3	.2	138 000	—
Control incubations (4)	<5 0		<0 4

Histamine like activity in / g base/g we weight
[b] No methylhistamine could be detected n this s mpl

Table V *Influence of control feces on inherent HA*
Incubation for 20–24 h at inherent pH

Case no	Feces wt in g: Control	Pat	HA[a] Before incubation	After incubation
1	1	1	30	20
1	3	1	30	1 7
1	5	1	120	850
1	14	3	28	11
1	5	1	18	8 5
1	5	2	35	10
3	5	1	22	2 0
3	15	1	22	<0 6

[a] Histamine like activity in μg base/g wet weight

Data between solid horizontal lines represent the same fecal specimen from patient as well as from control

Paper chromatography and autoradiography

Extracts from fecal samples with *high inherent histamine like activity* were submitted to chromatography (systems 1 and 2) and this disclosed that a double spot was present almost invariably The moving rate of the one spot corresponded approximately to that of authentic histamine di phosphate (which *occasionally* also gives a double spot in system 1 (24)) with the following rfs values system 1 approximately 0 08 (and 0 24) and system 2 approximately 0 40 Both spots could be localized by their UV fluorescence and were eluted without staining Both spots proved to contain biological activity which could not be differentiated from that of authentic histamine by parameters such as addition of antihistamine atropine or dihydroergotimine to the biological bath during assay

A spot that on the basis of moving rates could not be differentiated from *N* acetylhistamine was most often present in patients feces

Both conventional and labelled histamine added to patients feces were degraded but to a markedly less extent than by control feces A fraction with the same moving rate as *N* acetylhistamine evolved and so did an unidentified fraction with the approximate rfs 1) 0 58 and 2) 0 78 L histidine conventional and labelled was converted to hista m n (double spot) and to a much lesser extent to urocanic acid dihydrourocanic acid and to the afo ementioned unidentified spot Little unchanged L histidine was left In control feces the main

Table VI *Urinary and fecal excretion of histamine after oral L histidine loading (chronic pancreatitis)*

Case no		Control day	Loading 1st day	Loading 2nd day	Post loading 1st day	Post loading 2nd day
1	Free	90	58			
	Conj	7100	4000	—		
	HA[a]	125	18			
1	Free	29	96		19	42
	Conj	1100	3400	—	1500	1100
	HA	28	23 76[b]		13	37
3	Free	38	34	34		
	Conj	550	230	150		
	HA	13	—	19		
3	Free	100	93	240		
	Conj	1000	430	760		
	HA	24	65	100		

[a] Fecal histamine like activity in μg base/g wet weight
[b] *Two samples on same day*

metabolites were urocanic acid and dihydrourocanic acid The histamine spot was lacking or small and much of the L histidine remained unchanged after incubation (24)

No methylhistamine was detected in a fecal sample with high amount of generated histamine like activity

Oral sulfa treatment

During sulfa treatment in patient no 1 the fecal histamine like activity dropped to 17—the lowest observed control value in this patient being 47 μg/g—whereas the conjugated fraction in urine dropped to 340 the lowest control value being 820 μg/24 h Patient no 3 developed frequent diarrhea during sulfa treatment which therefore had to be discontinued In this patient only a slight decrease occurred in the fecal and urinary parameters

Oral L histidine loading

The two studies in patient no 1 showed opposite results both with regard to urinary free and conjugated histamine and with regard to fecal histamine (Table I) Patient no 3 developed frequent diarrhea during both studies the diarrhea starting less than 1 h after administration of L histidine so probably most of the amino acid was lost via the fecal route

Fecal pH

Fecal pH was measured in samples from altogether seven patients In five cases whose diagnosis was considered rather definite the mean pH was 6 0 (Table II) against a mean control value (26) of 7 13 ($P < 0\ 01$) In the whole series the mean pH was 6 3 It is noteworthy that in patient no 4 a fecal pH of 7 0 was accompanied by a conjugated histamine excretion of 87 μg/24 h whereas the fecal histamine like activity was 0 6 μg/g The corresponding figures accompanying a fecal pH of 5 6 were 240 μg/24 h and 6 9 μg/g

Urinary excretion of indican

Three samples from patients nos 1 and 3 were studied and a mean excretion of 65 mg/24 h was found (range 88–53 mg/24 h) In a control series (26) the values ranged from 41–113 with a mean of 62 9 mg/24 h

Urinary Output of Histamine in Patients with Various Other Disorders of the Pancreas

Acute pancreatitis

The results are presented in Table VII The pattern was entirely different from that of *chronic* pancreatitis in that none of the patients displayed increased urinary excretion of conjugated histamine The excretion of *free* histamine was increased in two patients and markedly so in patient no 15 in spite of medication with an antihistaminic drug Worthy of comment is also the increased fecal histamine like activity in this patient In our experience increased fecal histamine like activity is always accompanied by increased urinary conjugated histamine levels We have seen only one exception to this previously (27) and both in the previous and the present case it may be due to methodological errors the method used is not very accurate for estimating relatively low quantities of conjugated histamine in the presence of high quantities of free histamine since the conjugated fraction is calculated by difference (total histamine − free histamine = conjugated histamine) and the recoveries of both *N* acetyl histamine and histamine diphosphate vary considerably (23)

Cancer of the pancreas and mucoviscidosis

Normal excretion was found in the two patients with cancer of the pancreas whereas the two

Table VII *Urinary excretion of histamine in patients with acute pancreatitis*

Case no	Sex	Histamine in urine (µg base/24 h) Free	Conjugated	Drugs[a]	Urinary diastase
15	♀	250	32 (12[b])	Chlorpromazin digitalis, penicillin streptomycin phenergan®	256
16	♀	<2 0	14	Trasylol® chlorpromazin insulin penicillin cortison, meperidine	2048
17	♀	8	39	—	Three days after normalization
8	♂	49	34		10 4 on day before collection. 8 on collection day
19	♂	9	7	—	512 two days prior to collection 32 on day after collection
20[c]	♀	3	—	Tetracycline	4096
21	♀	7	18		Five days after normalization
—	♀	Trace	22	Penicillin, streptomycin	51

Drugs given during or just before the collection of urine
Histamine-like activity in feces µg base/g wet weight.
This patient also had cholecystitis.

children with mucoviscidosis showed moderately increased excretion of conjugated histamine (Table VIII)

DISCUSSION

The present study confirms the previous observation (27) that in patients with chronic pancreatitis increased urinary excretion of *conjugated histamine* frequently coexists with increased fecal output of histamine like activity In cases in which the diagnosis was considered rather definite the incidence of augmented excretion was high

As far as the *free fraction* in urine is concerned increased excretion was observed in some cases with increased excretion of conjugated histamine

The observed features may serve in the differential diagnosis of steatorrhea In idiopathic 5HIAA excretion is elevated (11 14) whereas conjugated histamine excretion in urine mostly is normal In chronic pancreatitis HIAA excretion in urine is normal (11 14) but conjugated histamine excretion in urine is evated and so is the *histamine like activity in* feces It should be stressed that as for histane there will be exceptions With Arjona s et al s method (3 22) the estimation of histamine like activity in the feces is a rather simple procedure

The close correlation between the excretion of conjugated histamine in urine and histamine like activity in feces points to a cause and effect relationship between the two phenomena The following reasoning may have a bearing on what is the *primary* abnormality in this sequence of events When histamine is ingested (1) or administered into the gastrointestinal tract (25) the following pattern is found the fecal output of histamine rises and so does the urinary output of conjugated histamine whereas the output of free histamine does not increase significantly (the conjugated/free histamine ratio increases markedly) The similarity of this pattern with that observed in chronic pancreatitis indicates an intestinal origin of the histamine in this disorder

Table VIII *Urinary excretion of histamine in patients with cancer of the pancreas and mucoviscidosis*

Case no	Sex	Diagnosis	Urinary histamine (µg base 24 h) Free	Conjugated
23	♀	Cancer of the pancreas	[illegible]	5
24		Cancer of the pancreas	5	[illegible]4
25 A	♂	Muco iscidos s	5	5
25 B		Mucovisc dosis	5	0
6	♀	Muco sc dosis	9	36
Control alues in 8 children <9 y mean and range (–7)			5 3 (– 11)	5 1 (0–10)

The ch ldren aged 4 5 (no 25) and y (no 6)

The following possibilities merit consideration to explain the presence of increased quantities of histamine (or N acetylhistamine) intraluminally in the intestine in this disorder 1 Ingestion of histamine 2 Release of histamine into the intestinal lumen 3 Production of histamine intraluminally 4 Decreased breakdown of intraluminally formed histamine Furthermore ingestion of N acetyl histamine must be considered

There is little to support the idea that histamine or N acetylhistamine preformed in the food (2) might be the underlying cause since most of the patients were given regular ward diets during study Further patient no 1 was studied for three successive days with a regular mixed diet without any change in the fecal or urinary excretion of histamine There is further evidence to refute the possibility of N acetylhistamine ingestion Ingestion of N acetylhistamine does not lead to increased fecal output of histamine like activity or conjugated histamine whereas our patients showed increased fecal excretion of these substances

Moreover the increased faculty of patients feces to form histamine (in in vitro studies) cannot be explained by an increased flow of histamine to the lumen of the gut be it by release of mucosal cells or by ingestion In that case these patients must have *two* abnormalities of histamine metabolism in the gut and that seems highly unlikely If decreased catabolism of histamine were to explain the inherent histamine like activity it is inferred that formation of histamine normally takes place in the lumen of the gut but that the formed histamine is inactivated before it can be detected This possibility seems unlikely since there is evidence that intraluminal formation of histamine in man is limited (24) The fact that ^{14}C histamine was catabolized rather efficiently by patients feces in in vitro studies tells against decreased catabolism as the only cause of the abnormally high histamine like activity in patients feces (For further discussion regarding the stability of inherent histamine in feces the reader is referred to (24)

By exclusion it seems logical to conclude that increased intraluminal formation of histamine (L histidine decarboxylation) seems to be the cause of the urinary abnormality in these patients Many intestinal bacterial species possess a potent L histidine decarboxylase The impetus of oral sulfa in patient no 1 may be due to reduction of such bacteria in the intestinal lumen The fact that only a reduction and not a normalization of urinary and fecal histamine was achieved with sulfa might possibly be ascribed to too low a dosage of sulfa since the quantities of feces in this patient amounted to 1 kg or more per day The incubation studies with sterile filtrates of feces also pointed to an important role for intestinal bacteria in this connection The most plausible explanation of the ability of control feces to reduce inherent histamine like activity in patients feces is that the effect is brought about by the bacterial flora as such

The bacterial decarboxylase has a low optimal pH 2 5–3 0 (9) In cases of steatorrhea due to chronic pancreatitis the fecal pH was decreased and this might facilitate histamine formation The pathogenesis for the increased intraluminal histamine formation may therefore be the following undigested protein (in increased quantities since creatorrhea is present ?) reaches the large bowel where the pH is low (lack of bicarbonate secretion by the pancreas?) Certain bacterial strains with the actual decarboxylase in their potential enzymic constitution will proliferate due to the preponderance of the substrate (16) L histidine is decarboxylated and histamine is formed The change in bacteria is probably secondary to the change in the intraluminal milieu

The metabolic aberration may thus differ somewhat from that in myotonic dystrophy in which the fecal pH was normal and the exocrine pancreas function seemed to be normal

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TWO METHODS FOR PRODUCING IN ANIMALS A MACROGLOBULIN SIMILAR TO THE RHEUMATOID FACTOR

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Abstract Two methods for provoking a haema glutinatin macroglobulin identical or similar to the Rheumatoid factor (RF) are described The macroglobulins were ob ( ) by injections of Diplostreptococcus agalactiae animals mainly white rats (*b*) by injecting into rats antibody to RF (anti RF) or the active fraction of antibody The anti RF was produced by immunizing bbits against RF Both (*a*) and (*b*) have given rise to tinating macroglobulins showing high titres (up 1 10^4) in the sheep cell test. The titre is much more table when usin method (*a*) Using method (*b*) the show sudden variations with sometimes total dis of the haemagglutinating macroglobulin.

The anti RF serum produced neutralizes the RF com in precipitation tests. The active principle of anti RF is a fraction showing a sedimentation constant of about 7 S i e exactly the same as that of the common γG Comparing the 7 S which has the property of neu tralizing RF completely in unheated state and the com mon γG (IgG) by immunoelectrophoresis, the first does not give rise to any precipitation line with anti-γG while of course the second provokes a typical line The RF neutralizing globulin seems to be a hitherto unknown 7 S globulin The same is true of the isolated heamagglutinat ing fragment of RF Details of the latter type of 7 S globulin were given in earl er papers

Thus, the active fractions both of RF and anti RF have been isolated and studied. They constitute two mem bers of the large 7 S family which certainly contains an enormous number of 7 S gamma globulins (γG or IgG) with different properties. As was pointed out earlier (10) there also exist a great number of d fferent macro-globulins.

some years Svartz has been engaged on trials in the experimental production of chronic poly thritis (5 15) Later these investigations were with experiments aimed at provoking n animals a haemagglutinating macroglobulin (7 8 9) identical or similar to the Rheumatoid fac (RF)

For this purpose injections of different types of bacteria were tried The most favourable results were obtained by using a strain of streptococcus B known earlier as Streptococcus agalactiae which is one of the main causes of mastitis in cattle The Streptococcus agalactiae is here called Diplostreptococcus agalactiae since even in fluid medium it has a pronounced tendency to appear in diploform (Fig 1)

It was stated that these cocci could fairly often be cultivated from the human throat and more often from RA patients than from healthy indi viduals In the course of the years many different methods of cultivating these bacteria were tried As regards some strains the addition of DNA seemed to promote the growth Our present meth ods of isolating agalactiae and of producing hae magglutinating macroglobulins will be described below

As was reported in this journal (14) a 7 S gam ma globulin γG or IgG was isolated from anti Rheumatoid factor serum (anti RF serum) produced in rabbits This IgG showed the capacity in non aggregated state of completely neutralizing the Rheumatoid factor The type of IgG in ques tion was called IgR in order to emphasize its connection with the RF and Rheumatoid arthritis (RA) The present paper will describe attempts to produce a macroglobulin of type RF by injections of anti RF into animals

MATERIAL AND METHODS

For the production of a haemagglutinating macroglobulin by means of bacteria the following method has been used dur ng the last two years

Diplococcus agalactiae (belonging to group B strepto-cocci (Fig 1)) obtained from the throat of patients with Rheumatoid arthritis, was first cultivated on 1 ga

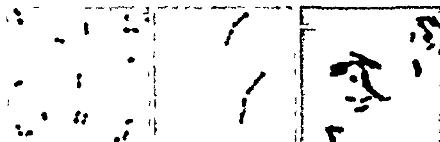


Fig 1 Microphotos of different shapes of Diplostreptococcus agalactiae (DSA) a coccus which is extremely pleomorphous

lactose broth or on blood agar or serum agar containing 1 galactose After 24–48 h at 34 C subcultures were made on blood agar plates with 1 galactose The cocci were obtained in a pure state by spreading on blood plates in series

A platinum loop from a 24–48 h blood agar culture was emulsified in 2 ml saline From this suspension 0.2–0 3 ml were injected intraperitoneally (sometimes intra venously) into white rats of type Sprague Dawley Simultaneously with the first or second intraperitoneal injection 0 2 ml of the same suspension was injected into or in the neighbourhood of the epiphyseal plate of a knee-joint This type of injection was repeated 1–3 times during the injection period which usually lasted for 6–8 weeks. For details see Table I If the haemagglutination titre was not satisfactory after 8 weeks the injections were continued The titre of haemagglutination was checked by the sheep cell test before and three or more times during the course of the injections Pigs and rabbits were treated in the same way but with higher amounts of bacterial suspension according to body weight

Table I *Example of the development of a haemagglutinating macroglobulin in rat (Sprague–Dawley) after injections of DSA*

	Haemagglut titre[a] (sheep cell test)	Injections (strain E 7)
1966		
June 1	0	Intraperit
June 2		Intraperit and epiph plate
June 7		Intraperit
June 9		Intraperit and epiph plate
June 11 14 17		Intraperit
June 20		
June 21	0	Intraperit and epiph plate
July 5	1 16	Intraperit
Aug. 30	1 64	
Oct 18		Intraperit
Nov 11	1 256	
Nov 30	1 512	
1967		
Jan 9	1 512	
March 14	1 256 (haemolys)	
April 4	1 512	
April 20		Epiphys plate
Sept. 11	1 512	
1968		
Febr 2	1 256 (haemolys)	
March 11	1 128 (bad condit treatment with vitamins)	
May 31		Died

[a] Considered negative up to a titre of 1 32.

The other method for producing RF like macroglobulins was quite different The Rheumatoid factor (RF) was isolated from Rheumatoid arthritis serum by a method published in J.A M A 1965 (10) and elsewhere The isolated RF was injected into rabbits twice at an interval of about three weeks using the common method for producing antibodies An antibody with high titre and completely neutralizing the RF was usually found after three–four weeks The anti RF serum did not contain any RF at least not at the time when the rabbits were killed by bleeding. The serum obtained was used for several purposes In this paper one type of experiments will be described The anti RF serum was injected into rats and rabbits (see Results) The injections were repeated twice or more The optimal number of injections has not yet been found but it seems necessary to give at least three injections otherwise the typical macroglobulin disappears rapidly

Sera from the injected rats and rabbits were investigated for the presence of RF like macroglobulins by sheep cell tests and immunoelectrophoresis Experience showed however that rabbits could not be used for this purpose

The next step consisted of trials in isolating the fraction of the anti RF serum which gives rise to neutralization of RF (see the aforementioned article (14) in Acta med scand) As reported earlier the RF neutralizing factor was mostly found in the supernatant from the cold precipitate of anti RF serum and was purified by chromatography The RF neutralizing fraction of anti RF was injected into rats in the same way as the original anti-RF serum



Fig 2 X ray pictures of the arthritic knee joints of a rabbit which was given injections of DSA Magn 1 .

RESULTS

As already mentioned it has proved possible to produce a haemagglutinating macroglobulin in animals particularly white rats by injections of Diplostreptococcus agalactiae (DSA) The haemagglutinating macroglobulin has the same properties as the Rheumatoid factor e g as regards the sheep cell test (Waaler Rose) sedimentation constant on ultracentrifugation and immunoelectrophoresis

The noteworthy circumstance was discovered that while a marked arthritis often progressive during a year or more appeared in rabbits (Figs 2–3) it was found almost impossible to provoke a haemagglutinating macroglobulin in this type of animal The highest titre obtained with Waaler Rose s test in experimental rabbits did not amount to more than 1 64 i e only a sligthly positive reaction As regards rats the reverse conditions were found the arthritis was often slight but the titre of haemagglutinating macroglobulin high e g up to 1 1024 In some rats however signs of pronounced arthritis occurred (Fig 4) Whether the haemagglutinating factor produced in rats is identical or whether small dissimilarities exist in comparison with the RF has not yet been established with certainty For this purpose the molecular structure has to be studied

Some pictures may illustrate the production of RF by injection of DSA Table I has already been referred to on page 136 Table II shows the course of bacterial injections and the results of the sheep cell test in one of 120 rats This table also shows that it was necessary to give extra injections when the haemagglutination titre showed a tendency to drop Table III demonstrates an experiment in



Fig 3 Same rabbit as in Fig. 2 X ray of one of the shoulder joints. Magn 1 2



Fig 4 Arthritis in a white rat injected with DSA

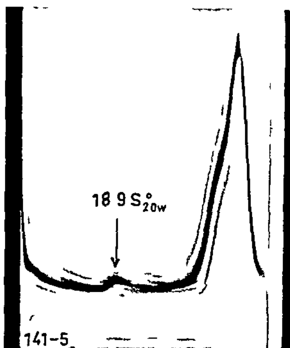


Fig 5 Ultracentrifugation of serum from the rat in Table III Nov 9 1960 The haemagglutinating macroglobulin had an S-value of 18 9

which cortisone injections were given at the same me as intraperitoneal injections of the cocci As uld be expected no influence on the production of RF was found Fig 5 shows ultracentrifugation on the serum of this rat and Fig 6 granulation tissue in the neighbourhood of the joint capsule These changes have here the character of vasculitis and perivasculitis

An X ray picture of an affected joint of a rat has already been shown in Fig 4

As reported above the experiments from earlier years were performed mainly on rabbits and often gave rise to an advanced and progressive arthritis (Fig 2) Fig 7 shows granulations in the joint capsule from a rabbit which had received injections of Diplostreptococcus agalactiae During a period Svartz had in her animal department several rabbits which had had chronic progressive arthritis during one year or more Later when the interest was focused rather on the possibility of producing a haemagglutinating macroglobulin by means of bacteria investigations were made also on other animals We already knew at that time that guinea pigs were out of the question since their sera mostly give rise to a positive Waaler Rose s test Investigations were made on white rats pigs dogs

Table II *Production of haemagglutinating macroglobulin in rat by another strain of DSA than in Table I*

	Haemagglut titre (Sheep cell test)	Injections (strain Ek)
1966		
June 1	0	Intraperit
June 2		Epiphys plate
June 7		Intraperit
June 9		Intraperit and epiph plate
June 11 14 17		Intraperit
June 21	1 32	Intraperit and epiph plate
July 5		Intraperit
Aug 30	1 128	Intraperit
Sept 21		Intraperit
Oct 4	1 64–1 128	
Nov 21	1 32 (haemolys)	
Nov 30		Intraperit
1967		
Jan 17	1 1024	
March 14	1 256 (haemolys)	
April 4	1 256 (haemolys)	
April 20		Epiphys plate
Sept 11	1 512	
Sept 14		Killed (5 ml serum obtained)



Fig 6 Granulation tissue in the neighbourhood of a joint capsule of the rat in Table III

sheep etc It was soon found that rats gave the best results

Up to 1964 about 30% of the rats showed a positive sheep cell test after injections of agalactiae During the last three–four years the percentage has after methodological modifications amounted to between 50–60 The result of the sheep cell test has as in human beings been considered negative up to a titre of 1 32

A few comments should be made concerning the different types of injections which have been tried intraperitoneal intravenous subcutaneous injections and injections into the joint and in or in the neighbourhood of the epiphyseal plate of a kneejoint The reason why this latter method was tried was that we had found by autoradiographic investigations performed in collaboration with the Veterinary School that the Rheumatoid factor has a special affinity for the epiphyseal plate (10 12) It is too early definitely to judge the value of this method but we have the feeling that it has improved our results The intravenous injections

Table III *An experiment with intraperitoneal injections of DSA (Wall) in rat combined with cortisone injections during the first period*

	Haemagglut titre (Sheep cell test)	Injections (strain Wall)
1959		
Nov 25	Neg.	40 intraperiton injections were given During the first period cortisone inj were given simultaneously
1960		
Jan 4	1 32	
Jan 25	1 3_	
Febr 6	1 32	
March 3	1 64	
March 21	1 512	
April 5	1 256	
April 25	1 256	
May 16	1 128	
June 7	1 64	
June 0	1 1 8	
Aug 8	1 1 8	
Sept 13	1 256	
Nov 9	1 256	
Nov 26		Died

are not superior to the intraperitoneal Our earlier and present experiences have shown that subcutaneous and intramuscular injections are less effective

As has been reported in J A M A and elsewhere (10 11 13) the entire RF is not needed for the production of a positive sheep cell test (Svartz) but only a small fragment of it with a sedimentation constant of about 7 S (Fig 8) In spite of the fact that the haemagglutinating fragment has the same sedimentation constant as the common 7 S or γG it has quite different properties otherwise A description of the haemagglutinating fragment (subunit) of the RF has appeared in Bull Acad nat Med (Paris) 1967 (11) and elsewhere

It was of course important to know whether the experimentally produced haemagglutinating macroglobulin in rats contained a similar haemagglutinating 7 S-fragment to that of the human RF Our investigations showed this to be the case

It should be mentioned that considerable variations have occurred in the rapidity with which the haemagglutinating macroglobulins appear in animals This is in analogy with the conditions in human beings and is not surprising since it is a question of biological changes i e changes of the enzymatic processes in the organism provoking a new type of gammaglobulin metabolism

Speaking of the metabolic changes studies of the NH end groups of the Rheumatoid factor

Table VI *Production of macroglobulins indistinguishable from the Rheumatoid factor (RF) by injections of anti-RF serum or a 7 S fraction of it called IgR*

	Haemagglutination titre (sheep cell test)[a] after injections into rats (Sprague–Dawley) of the following materials				
	Anti-RF serum (rabbit) to rat A	Normal rabbit serum to rat B	RF neutralizing 7 S globulin called IgR to rat C	Isolated 7 S globulin from human gamma globulin to rat D	Inject (0.25–0.5 ml) intraarticularly or in the neighbourhood of the epiphyseal plate of a knee joint
1968					
Jan. 15	0	0	0	0	A B C
Jan. 18					A B C
Jan. 30					A B C
Febr. 5	1:64	0	0		
Febr. 12					A B C D
Febr. 13					D
April 1	1:128	1:8	1:64	1:8	
April 29	1:32	0	1:32	1:16	
April 30					A B C D
May 13	1:16	1:16	1:64	1:16	
Aug. 19	1:512	1:16	1:64	0	
Nov. 25		dead			A C D
1969					
Jan. 20	1:1 S		1:128	0	

[a] Considered negative up to a titre of 1:32.

serum of the rats. This rat serum containing the RF (or a very similar substance) gave rise to a positive sheep cell test often with high titre (i.e. up to 1:1024).

We found great and hitherto unexplained variations in the appearance of haemagglutinating macroglobulins provoked by injections of anti-RF as may be seen from Tables V and VI. It should be added that rabbits reacted unfavourably to the injections of anti-RF. In order to stress the connection between the RF and the 7 S fraction of anti-RF serum we have tentatively called this fraction IgR.

DISCUSSION AND CONCLUSION

It may be considered to be established that macroglobulins can be provoked through the action of bacteria. This is particularly clear as far as the haemagglutinating macroglobulins of the Rheumatoid factor type are concerned (7, 8, 16) and had been proved already in 1954–55 (Svartz). In our experience injections of Diplostreptococcus agalactiae constitute the best method hitherto for this purpose. At the same time arthritis is often produced. It has to be mentioned that Diplostreptococcus agalactiae is sometimes hard to differentiate from enterococci even on typing with specific sera. In fact we called our strains "enterococci" during the first period.

As regards the development of arthritis, many investigators have provoked joint diseases by other means than those employed by us. Advanced experiments have been performed for instance with Freund's adjuvant (1, 3, 4). In connection with these experiments an RF-like macroglobulin does not seem to have arisen.

From some earlier observations it was obvious that not only agalactiae but also other bacteria are able to alter the protein metabolism resulting in the formation of a haemagglutinating macroglobulin. Rheumatic fever has probably nothing to do with agalactiae as it is caused by haemolytic streptococci of group A. In 1953 (6) we showed that a transitory positivity of the sheep cell test is rather common in rheumatic fever. The positive sheep cell test appeared after the patients had had the disease for 4–6 weeks. In this connection it is interesting that Fudenberg and Kunkel (2) observed a positive sheep cell test in septic endocarditis.

Some years ago a strain of the group A strepto ..i was received from Russell Cecil which he been studying in collaboration with R Lance eld These cocci gave rise to a transient positivity n rats similar to that in rheumatic fever

Other bacteria as well have been found to pro- oke a positive sheep cell test but usually with ow titre

When discussing these matters it has to be borne i mind that some investigators including our p made the mistake initially of not completely osorbing the heterophile haemagglutinins before ig the sheep cell test This was the case in ur earliest experiments with injections of cocci into rabbits Thus it sometimes happens that the eterophile agglutinins are difficult to absorb and ive rise to a strongly positive sheep cell test This act is now well known Therefore an incomplete osorption of h terophile haemagglutinins is an which hardly occurs in our days

The question whether Diplostreptococcus agal ctiae might be the aetiological agent of Rheuma arthritis (RA) has been discussed by Svartz arlier No conclusive proofs exist that this is the ase But it is interesting that the bacteria in ques ion are cultivated more often from the throat stools of patients suffering from RA than m healthy individuals This fact is of course proof that DSA is involved in the aetiology nd pathogenesis of RA A large material would e needed for instance in order to state the dif between RA patients and healthy indi iduals as regards the presence of DSA in the oat (also confirmed by V Houba M D Prague) It is a hitherto unexplained circumstance that a ..lutinating macroglobulin though in a ther low percentage (except in disseminated lu is found in rather many diseases of dissimilar ology We have for instance sometimes found positive sheep cell test in liver cirrhosis and ronic pancreatitis (13) The haemagglutinating acroglobulins in these conditions may be pro- uced by some other types of bacteria than in RA by viruses or toxins

The macroglobulins giving rise to haemaggluti tion differ from each other in sedimentation tant etc but their haemagglutinating frag seem to be identical or very closely related hus the macroglobulins as such are not needed a positive sheep cell test but as we have it is necessary that they contain a specific haemagglutinating fragment (subunit) As reported before this haemagglutinating fragment has been isolated (Svartz) Like the so called γG or IgG it is a gammaglobulin with a sedimentation constant of 6–7 S but it has other biological properties One of the most important differences is that the haemagglutinating fragment does not react with common anti γG It reacts strongly with anti kappa serum but it gives also a weak precipitation with anti lambda

As point d out above we have been able to produce a haemagglutinating macroglobulin by a method quite different from the bacterial one After having produced an antibody against the isolated RF we injected this antiserum into rats A haemagglutinating macroglobulin with high titre in the sheep cell test and showing the same properties as the Rheumatoid factor was produced However this haemagglutinating macroglobulin often disappeared from the blood of the rat if the injections were not repeated This is in con tradiction to the results with bacterial injections In the latter case the haemagglutinating macro- globulin often remains for several months without new injections (Tables I–III) The reason for this dissimilarity is possibly that when bacteria are injected they usually provoke a more or less pro- nounced continuous inflammatory process in the connective tissue which is easy to verify micro- scopically Injections of gammaglobulins do not provoke any distinct microscopical changes in the connective tissue

The fraction of the anti RF serum which reacts with RF has been isolated It is a 7 S globulin but not identical with the common 7 S (γG) While the common 7 S is able to neutralize the RF only to a certain extent (more pronounced when the ma terial is aggregated) the anti RF serum or its ac tive principle neutralizes RF completely This RF neutralizing fragment represents a new experience For reasons which have been mentioned above and in earlier paper (14) we have for the present called this RF neutralizing fragment IgR The study of this agent is our special concern at pres ent

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Acta med scand Vol 186 pp 145–147 1969

SCLEROSIS OF THE ARTERIES OF THE EXTREMITIES IN RHEUMATOID ARTHRITIS

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In a series of 370 patients with rheumatoid hritis and 438 controls radiological vascular changes re observed in 15 4 of the former and 3 4 of the tter The high rate of systemic manifestations in the heumatoid patients showing radiological changes may interpreted as evidence in favour of the view that eritis is an inducing factor in atherosclerosis

the last few years the various systemic ions of rheumatoid arthritis have at racted increasing interest Changes have been observed in many organs Vascular lesions which are the most common were described at the of the 1950 s by B Cruickshank This nvestigator found evidence of arteritis at autopsy n 25 of a series comprising 72 cases of oid arthritis (5)

Vascular lesions are mainly encountered in mall arteries arterioles and venules (3 11 12) The arterial lesions may cause unspecific ymptoms such as cold hands and Raynaud s enomenon Even cutaneous ulceration in the legs may occur (8) Acute arteritis in the mesen vessels may lead to intestinal ulcers and foration (1 6) More typical of rheumatoid rthritis is the occurrence of punctulate necroses in the finger tips and slight haemorrhages in the ilfolds (4)

MATERIAL AND METHOD

series consists of 370 patients w th rheumatoid hritis and 438 controls The controls were selected the pat ents at the Orthopaedic Hospital of the In alid Foundation. The controls showed no systemic disease no severe disabl ng lesions Patients who had been mmobilized for long periods were not included

Ordinary rad ographs were taken of the blood vessels in the hands arms feet and legs The rad olog cal method and the film quality were standardized All radiographs were examined by one and the same person, and only obvious vascular shadows were taken into account. Patients with diabetes mellitus or disturbances of the lipid metabolism were omitted from the series

RESULTS

Affected arteries were observed in 57/370 (about 15 4) of the patients with rheumatoid arthritis and in 15/438 (about 3 4%) of the con trols (Figs 1 2) A marked difference was ob served in respect of the age distribution

Calcium deposits were mainly encountered in the medium sized arteries of the lower extremities e g the dorsal artery of the foot and the posterior tibial artery and their branches In some cases calcium deposits were observed in the radial and ulnar arteries and the minor arteries of the hand (Figs 3 4)

Of the patients showing radiological changes 21 exhibited systemic manifestations i e renal cuta neous cardiac or pulmonary symptoms Five pa tients had malignant rheumatoid arthritis The duration of the illness was under five years in 18 cases with radiological changes and in these an obvious tendency towards high Waaler Rose titres was noted All patients except one showed satis factory mobility

DISCUSSION

In this study the ratio of arterial changes was significantly higher in patients with rheumatoid arthritis than in subjects not showing rheumatic disease In addition the lesions in question were found to develop earlier in life in the former

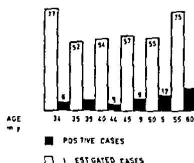


Fig 1 Radiological vascular changes in 370 patients with rheumatoid arthritis 57 positive cases about 15 4

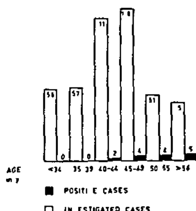


Fig Radiological vascular changes in 438 control patients 15 positive cases about 3 4%

The association of systemic manifestations and radiological vascular changes was striking This argues in favour of the view that the vascular changes were a consequence of rheumatoid arteritis It appears that the following three categories of arteritis may be distinguished

(*a*) Subacute lesions in small arteries of muscles such as have been described by Sokoloff et al (13) and similar vascular lesions in the heart muscles and nerve sheaths as described by Cruickshank (5) This subacute arteritis which is characterized by the accumulation of lymphocytes histiocytes in the vascular wall but is not associated with necrosis fibrinoid degeneration or aneurysm formation was considered by Cruickshank as typical of rheumatoid arthritis This view has not been confirmed by later investigations however

(*b*) Grave lesions resembling polyarteritis nodosa mainly encountered in the major vessels of the kidneys liver adrenals testes and central nervous system Such lesions have been described by e g Graef et al Levin et al Aronoff et al and Schmid et al (2 7 9 10) Microscopically acute inflammation medial muscle cell death and fibrinoid degeneration are seen in these cases

(*c*) Progressive and obliterating endarteritis in the arteries of the fingers as described by Bywaters (4) This category has very few features in common with type *b*

The question of whether arteritis in patients with rheumatoid arthritis induces calcium deposition is open and cannot be answered on the basis of this study It is also possible that the lesions in question are due to atherosclerosis In our opinion this is a less convincing explanation con

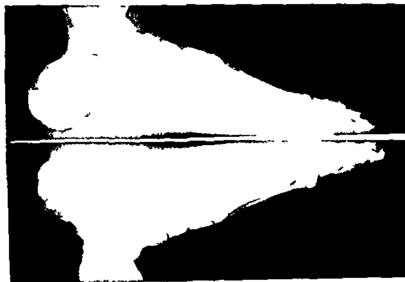


Fig 3 Radiograph of a 34 year-old woman with rheumatoid arthritis which began in 1961 Note marked sclerosis of dorsal artery of the foot



Fig 4 Radiograph of a 38 year old man with rheumatoid arthritis which began in 195 Note marked sclerosis of dorsal artery of the foot and posterior tibial artery

sidering the strikingly high rate of positive cases in the younger age groups in the present series

Immobilisation may be thought to predispose to early atherosclerosis In this series no correlation was observed between immobilisation and vascular changes On the other hand a positive correlation was demonstrated between arterial changes and other systemic manifestations

It seems obvious that rheumatoid arthritis causes connective tissue disturbances which either directly or via arteritis predispose to degenerative changes of the vascular wall resulting in fibrosis and calcium deposition which is visible on ordinary radiographs

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Congress Announcements

Le XVI Congrès National de la Tuberculose et des Maladies Respiratoires se tiendra à Bordeaux du 2 au 5 Avril 1970

Information Secrétariat du Congrès Laboratoire d Hygiène Faculté de Médecine Place de la Victoire 33 Bordeaux France

The Second International Air Pollution Conference will be held in Washington D C during December 1970

Program Co-chairman Professor Arthur C Stern Dept of Environmental Sciences and Engineering School of Public Health University of North Carolina P O Box 630 Chapel Hill N C 27514 U S A

Acta med scand Vol 186 pp 149–150 1969

QUANTITATIVE LIPID ANALYSIS OF HUMAN LIVER NEEDLE BIOPSY SPECIMENS

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Abst act A method is de cr b d for quantitative analysis of different lipid fractions from ordinary percutaneous liver biopsy specimens In a s ries of 37 liver biopsies a s gnificant correlation was found between free and esterif ed cholesterol and between triglycerides free and particularly esterified cholesterol respectively

Determination of liver lipids under basal condi tions and during different phases of therapy of hyperlipidemias could give valuable information as far as fatty liver the kinetics of serum lipids and the mode of action of therapeutic measures are concerned Yet the number of studies dealing with lipid analyses of needle biopsy specimens is limited (2 3 5) and furthermore data on detailed determinations of various lipid classes are lacking mainly because of the small amount of tissue avail e The combination of thin layer chromatog phy (TLC) and gas liquid chromatography (GLC) techniques for analysis of small amounts tissue lipids appears to be sensitive enough and in addition offers the possibility of determining tty acid composition of different lipid classes ication of this procedure to lipid analyses of liver biopsy specimens is reported in the present aper

METHODS

ercutaneous l ver biopsies were performed by the Mengh ni technique (8) Additional samples were taken at au opsy or from rats maintained on an ordinary laboratory diet. Immediately after performance of the biopsy the pecimen was placed in a preweighed glass stoppered tube and weighed The tissue was then homogenized by a Pot ter Elvehjelm type apparatus in chloroform methanol (1) to which C-cholesterol (free and esterif ed) and β s tos terol (free and esterif'ed) were added for recovery tests The chloroform phase was evaporated and l pids were separated by TLC on silica gel G (6) de eloping chroma toplates with ethyl ether heptane and acetic acid (40 10 1) Cholesterol esters tri lycerides FFA cholesterol and phosphatides could be easily recovered from the plate for qualitative and quantitative analysis after spray ing with rhodamine Cholesterol and cholesterol esters (saponified in alkaline ethanolic solution) were determined as their TMSi-ethers by GLC using 5 α cholestan as an internal standard (9) Correction for the losses taking place durin the procedure was made by the recovery of either C-cholesterol or β sitosterol Triglycerides were measured by the carboxyl ester reaction (10) or by GLC of fatty acid methyl esters (5) prepared by methylating tri lyceride fatty acids with methanol HCl after preceding saponification Trimargarine was used as an internal stand ard The loss of triglycerides during the isolation procedure was assumed to be the same as that of cholesterol frac tions Phosphatides were determined colorimetrically (1) after digestion of the phosphatide-containing silica gel area of the chromatoplate or by the carboxyl ester reaction after methylation of fatty acids released from phospha tides by saponification GLC of fatty acid methyl esters was carried out using a 15 diethylene glycol adipate column The water phase of the homogenization mixture was used for determination of protein (4)

RESULTS AND DISCUSSION

The recovery of cholesterol and cholesterol esters was 90–98 ⁄ according to the recovery of added radioactivity or β sitosterol Although the liver tissue contained trace amounts of endogenous plant stero's a mixture of free and esterified β sitosterol in relatively large amounts appeared to be an ideal internal standard because this sterol behaves like cholesterol and could be determined in the same GLC run as cholesterol Table I shows that although the weight of the liver pieces from a cadaver and a rat ranged in two series of six determinations from 4 mg to 41 mg and from 4 5 mg to 19 8 mg respectively the standard deviation of the mean for free and esterified cho lesterol and for triglycerides was relatively small

Table I *Replicability of cholesterol and triglyceride determination from liver of a human cadaver and a rat*

Mean ± standard deviation (s d) of six determinations

Source	Range of tissue weights (mg)	Cholesterol Free (mg/100 g)	Cholesterol Ester (mg/100 g)	Triglycerides (g/100 g)
Human	4–41	233±21	48±4 0	0 74±0 02
Rat	4 5–19 8	209±18	48±4 9	0 63±0 06

Thus the methodological error of the entire procedure including weighings after correction of the recovery is within the limits of ±10% even in the case of small tissue pieces Similar results were obtained for phosphatides Determination of triglycerides appeared to be more accurate by GLC than by color reaction particularly in small tissue specimens with low triglyceride content Variation of the results was smaller on the basis of wet weight than of the protein content

In a series of 37 liver biopsies carried out mostly on obese and hyperlipidemic subjects there was a significant correlation between free and esterified cholesterol ($r = 0\ 75$) In addition as illustrated in Fig 1 liver free and particularly esterified cholesterol showed a significant correlation to triglycerides ($r = 0.57$ and 0 70 respectively) indicating that the fatty liver contains also increased amounts of cholesterol In agreement with other studies on liver needle biopsies (2 3 5) and biopsies taken by surgery (7) triglyceride concentration shows a wide range of variation being almost 100 fold in the present material (0 26–21 6 g/100 g) Most of the values (Fig 1) are below 5 g/100 g which app ared to be the limit for the microscopic detection of fatty infiltration Free and esterified cholesterol which have not been earlier determined separately from liver biopsies showed less marked variation being for free cholesterol only about 4 fold (142–620 mg/100 g) and for esterified cholesterol about 10 fold (30–378 mg/100 g)

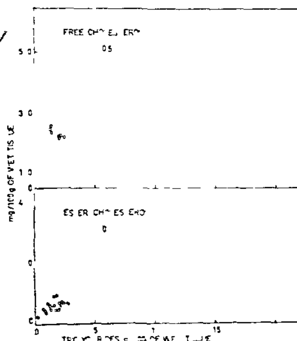


Fig 1 Correlation of free and esterified cholesterol to triglycerides in liver needle biopsy specimens of 37 human subjects.

ACKNOWLEDGEMENTS

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Acta med scand Vol 186 pp 151–155 1969

HERPES SIMPLEX HEPATITIS IN AN ADULT

A Case Report with Virological and Electron Microscopical Examination at Autopsy

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bstract A case report is given of a man with bronchial thma who fell ill in a stomatitis He was treated inter lia with antibiotics and corticosteroids Haematemesis ielena, jaundice anuria and circulatory insufficiency eveloped and the patient died Autopsy revealed hepa itis erosive gastritis with gastrointestinal bleeding and hock kidneys Histologically the hepatitis was consistent th a herpes simplex hepatitis Herpes simplex virus was solated from the liver

Almost all cases of herpes simplex hepatitis de cribed have been encountered in newborns with eneralized herpes infection (for references see 5) ı older children there is evidence of infection of he liver only in a few cases (1 8 9) To our nowledge herpes simplex hepatitis has not been in adults

We have encountered a case of herpes simplex epatitis in an adult with a fatal outcome Viro ogical and electron microscopical examinations performed on material obtained at autopsy he present paper will give a brief description of he case

CASE REPORT

he patient was a male factory leader born in 1907 with hial asthma since 1970 possibly of infectious origin In 19 9 and 1960 he was treated with Acton® Ferring ACTH) 10 IU every three days and adrenalin spray rom the end of 1960 Acton was chang d to Kena ort® Squibb (fluoxiprednisolon) 4 mg a day which taken rather regularly

On November 7 1967 the patient developed pains in s throat headache and a rectal temperature of about 39 C He had no asthma symptoms and took no Kena He was treated with Tetradecin Novum® Astra tetracycline) 0.25 g × 4 for one day followed by Doktacillin® t blets Astra (ampicillin) 0 5 g × 4 The temperature remained elevated and he was admitted to the County Hospital on November 10 He was then in a fairly good condition The tongue was white and in the tonsillar regions there were white papules surrounded by red zones The treatment with Doktacillin continued with the same dosage He was also given Kenacort 2 mg × from November 15 4 mg × 2 and Bamyl® Hassle (acid acetyl salicyl 0 5 g calc carb 0 15 g and acid citr 25 mg per tablet) 2 tablets × 4 The temperature fell to about 38 C Due to his aching stomatitis he ate almost nothing and gradually deteriorated

On November 17 a rather large haematemes s occurred followed by bloody diarrhoea An enlarged liver was noticed Urine became sparse and did not increase despite treatment with 300 ml Mannidex® Pharmacia (15 mannitol) He was also given 1000 ml Inverdex® Pharma ia (sacchar invert 100 g aq steril ad 1000 ml)

On November 18 the patient was transferred to the Department of Infectious Diseases University Hospit l Uppsala He was deep-breathing and his tongue and most of the oral cavity were covered with yellow crusts The systolic blood pressure was 125 mm Hg The liver was slightly enlarged and tender There were still small amounts of urine His electrolytic balance was greatly disturbed (see Laboratory data) He had repeated bloody diarrhoea and the systolic blood pressure fell to 80–85 mm Hg Th temperature was around 38 C On this day he was given Inj Doktacillin® 0 5 g × 3 Inj Solu Glyc® Erco (hydrocortison) 300 mg, 1000 ml Inverdex 50 ml Mannidex 450 ml blood Inj Calcium Sandoz® totally correspond ng to 90 mg Ca 15 g Resonium® Winthrop (sodiumpolystyren su phonate) and Inj Ara m ne® MSD 50 mg in 500 ml Inverdex

On November 19 the urine secretion totally ceased the blood pressure remained low and the pat ent died

Laboratory Data

No 11 Hb 150 g 100 ml White blood cell count (WBC) 4300 mm with 81% polymorphonuclear neutrophils, 1% basophils 16 lymphocytes and 2% mono

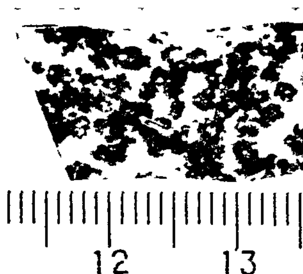


Fig 1 The surface and the interior of the liver is dotted with haemorrha ic necroses Measurements shown in cm

cytes Serum Na 131 mEq/1 (normal 134–146 mEq/1) serum K 3 6 mEq 1 (normal 3 6–4 6 mEq/l) serum Ca *4 8 mEq l (normal 4 –5.2 mEq/l) serum Cl 95 mEq/l* (normal 97–108 mEq l) serum protein 6 6 g/100 ml (normal 6 1–8 1 g 100 ml) The urine contained albumin but only few cells Serum creatinine 1 5 mg 100 ml (nor *mal 0 6–1 2 mg 100 ml)*

Nov 14 Serum bilirubin 0 6 mg 100 ml (normal 0.2–1 mg 100 ml) thymol turbidity test extinction value 0 05 (normal < 0 1) serum alkaline phosphatase 6 Bessey Lowry units 100 ml *(normal 0 9–2 5 units/100 ml) serum* glutamic pyruvic transaminase (SGPT) 500 units (normal < 35) Cold agglutination test neg

Nov 16 Cerebrospinal fluid total globulin 76 mg/100 *ml (normal 20–50 mg 100 ml)* Mastix neg No cells Serum creatinine 4 6 mg 100 ml

Nov 17 Thrombocytes 103 000

Nov 18 Hb 15 4 g 100 ml Haematocrit 46 WBC 5400 with 87.5% polymorphonuclear neutrophils, 0 5% basophils, 4 lymphocytes 3.5%, monocytes, 3 5% plasma cells and 1 metamyelocytes Thrombocytes 88 000 ESR 12 mm 1 h Serum creatinine 10 3 mg 100 ml serum bilirubin 0 mg 100 ml thymol turbidity test 1 MacLagan units (normal < 3) serum alkaline phosphatase 8 7 units/100 ml serum glutamic oxaloacetic transaminase (SGOT) 6 50 units (normal < 35) SGPT 1800 units Serum Na 121 mEq 1 s rum K 6 mEq 1 serum Ca 3 9 mEq 1 serum Cl 9 mEq 1 plasma HCO 19 mEq 1 (normal 18 5–24 5 mEq/l)

Nov 19 Hb 13 1 g 100 ml serum creatinine 14 0 mg 100 ml, serum Na 110 mEq 1 serum K 5 1 mEq 1 serum Ca 2.6 mEq/l serum Cl 80 mEq 1 serum protein 5 1 g/100 ml

Canadida albicans was cultured from urine and faeces on Nov 18

Autopsy

Macroscopical examination

Autopsy was performed 46 hours after death Situs in versus was found but no other malformations

On the skin no vesicles or crusts were seen On the tongue the palates and the buccae there were several white papules 3–5 mm in diameter often crusted or ulcerated.

The lungs showed slight emphysema but no bronchitis or bronchiectasis They were of normal size The heart weighed 530 g but there was no cor pulmonale

The liver was studded with haemorrhagic necroses, 3-6 mm large over the outer and cut surfaces (Fig. 1) It weighed 2050 g and was very swollen

The spleen was heavily congested and weighed 215 g The kidneys were slightly swollen and had a pale cortex They weighed 205 and 190 g In the pelvis of the right kidney there was a coral stone partially obstructing one calyx The urine b adder showed slight cystitis The prostate was *nodulated and of benign appearance*

The stomach and the small intestines had bloody con-

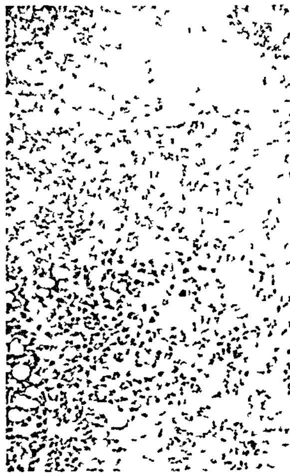


Fig 2 Histological section of the liver Necrotic areas are seen in the upper and lower part of the picture There is a slight fatty change in the preserved parenchyma Haematoxylin-eosin × 90

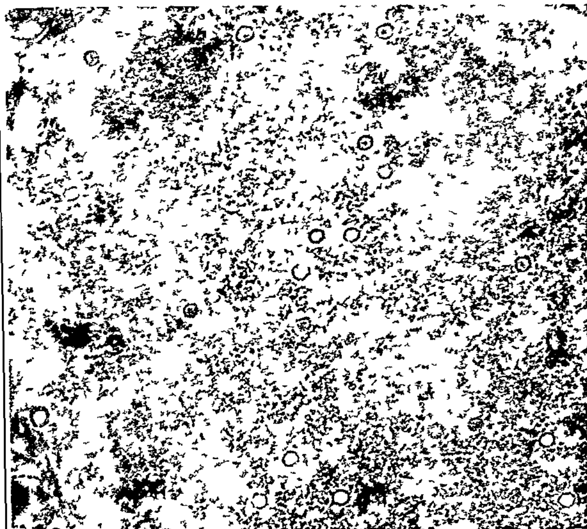


Fig 3 Electron micrograph of section of liver cell nu cleus Virus particles with hollow and dense cores are seen Uranyl acetate lead citrate × 47 000

tents There were several small shallow eros ons in the stal part of the stomach

No other relevant findings were made

croscopical examinat on

ne buccae showed shallow ulcers with fibrin and poly ıonuclear leucocytes. No giant cells or inclusion were found

The liver showed a large number of often confluent ıorrhagic necroses (Fig 2) They were situated in all s of the lobules and often covered more than one obule Many erythrocytes and a moderate and varying er of polymorphonuc ears were found within the roses The demarcation towards the vital tissue was ther sharp In this zone occasional liver parenchymal ells were found with intranuclear inclusion bodies In he peripheral parts of the necrotic areas necrotic nuclei taking the same colour as the inclus on bodies were often seen A few liver cells had –3 nuclei. Occas onal paren chymal cells had a ballooned appearance The preserved part of the liver parenchyma showed a slight fatty chan e

The stomach showed erosions It was too autolytic for re iable examination The kidneys were autolytic A large number of hyaline and haemoglobin casts were found

The other organs examined heart, lungs pleen pan creas and intestine showed no findin s of nterest.

Elect on m croscopy of the liver

The tissues had been f xed in 10 formaldehyde for a few days They were postfi ed in glutaraldehyde and osm c ac d according to Hubner ( ) and embedded in Epon Sect ons were cut with an LKB ultram crotome sta ned with uranyl a etate lead citrate and examined n a S e mens Elmiskop I electron m croscope

Virus particles were found in a few liver cell nuclei often in large amounts The particles consisted of a ring with hollow or dense cores (Fig 3) In the cytoplasm virus-like particles were only occasionally encountered Their viral nature was questionable No typically enveloped particles were seen

Virological Examination

For virus isolation supernatants of 10 % homogenates of liver spleen kidney lung and blood clot from the heart were prepared and inoculated into cultures of BS C 1 cells (a stable line of monkey kidney cells) No cytopathic changes were seen after the inoculation of the specimens from the spleen, kidney lung or blood clot The liver specimen however caused cytopathic changes characteristic of herpes simplex virus The isolate was typed by neutralization with antiserum to a known strain of herpes simplex virus (strain E263 isolated from a patient with stomatitis)

Serum, taken at autopsy was tested for neutralizing antibodies using 100 TCD_{50} of virus The titre against the strain E263 as well as the strain isolated was 1/16 The serum could not be used in the complement fixation test due to its haemolytic effect

Summary of Autopsy and Virological Findings

Stomatitis Herpes simplex hepatitis Shock kidney " Erosive gastritis with gastro-intestinal bleeding

DISCUSSION

The 60 year-old patient had thus had bronchial asthma for 47 years which had been treated with ACTH and corticosteroids for the last eight years He developed a stomatitis the aetiology of which was not known

He was treated with inter alia antibiotics and corticosteroids but deteriorated rapidly Haematemesis melena anuria and jaundice developed and the blood pressure was falling The patient died twelve days after the beginning of the illness

Autopsy revealed stomatitis multiple haemorrhagic necroses in the liver erosive gastritis with large gastrointestinal bleeding and shock kidneys In the liver intranuclear inclusion bodies were found under the light microscope and virus particles in the electron microscope Herpes simplex virus was isolated from the liver The macroscopical light microscopical and electron microscopical picture of the liver was consistent with that described for infant with herpes simplex hepatitis (4) However it was not a giant cell hepatitis, which has been described in some cases of herpes simplex hepatitis in newborns (3)

The hepatitis might have been the result of a primary or a recurrent infection with herpes simplex virus At the age of the patient a primary infection is uncommon but cannot be excluded. If the stomatitis was caused by herpes simplex virus a primary infection is even the most probable alternative It is argued that herpes simplex stomatitis seldom if ever recurs (7)

The development of the hepatitis might have been due to the corticosteroid treatment as this may be deleterious in viral infections The erosive gastritis may also have been a sequel of the corticosteroid treatment but an effect of the salicylates (Bamyl) may be considered (6) Liver injury might also have been a factor in the development of the erosive gastritis as increased levels of SGPT and serum alkaline phosphatases were present on November 14 three days before manifest signs of gastrointestinal bleedings The possibility also exists that the gastritis was caused by the virus Lesions in the stomach have been found in cases of generalized infection with herpes simplex virus (9) In this case the stomach was too autolytic for reliable examination

The voluminous gastrointestinal bleedings and the extensive liver necroses were considered to be responsible for the falling blood pressure and consequent anuria It was impossible to decide which of these two factors was the predominating one for the fatal outcome A pure bleeding shock should have been associated with a lower haemoglobin value

ACKNOWLEDGEMENTS

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QUINIDINE PRIOR TO DIRECT CURRENT COUNTERSHOCK —A MEDICAL HAZARD

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Abstract The author has previously discussed a possible relationship between post conversion arrhythmias and quinidine administered prior to a countershock. A further evaluation of the suspected relationship is now possible after a more extensive follow-up Evidence is provided that quinidine as well as digitalis should be avoided immediately before direct current conversion The reason is the highly increased risk of post conversion arrhythmia

The purpose of this communication is to stress the importance of avoiding quinidine prior to direct current countershock (DC shock) The reason is the increased risk of serious ventricular arrhythmias The author has previously discussed a possible relationship between quinidine and/or digitalis prior to the shock and these arrhythmias (3) Since that report was prepared at the beginning of 1967 a further evaluation of this suspicion is possible

MATERIAL AND METHODS

Our series of patients with atrial fibrillation treated by DC shock has been presented earlier (3 4)

The method of conversion and of anesthesia has already been described (3 4 6) With regard to drugs and the countershock procedure the rules have changed in our ... since we started to use the DC technique in ...

Since January 1966 we have discontinued digitalis prior ... the shock (3) Since September 1966 we have not ... quinidine until after the shock. Before then the ... had a serum level between 4–7 mg/l at the time ... the countershock, considered as normal maintenance ... according to our method (8) (Sixty-six ... in 1967–1968 received 0.5 g procainamide 2–3 ... before the shock. These patients were included in ... clinical investigations (1 ?))

Thus the material is divided into three main groups ...ng to the different schedules of premedication ...I)

RESULTS AND COMMENTS

The occurrence of post conversion arrhythmia among the different groups is presented in Table I Complete absence of ventricular tachycardia and fibrillation is noted in the group in which digitalis was discontinued and quinidine not administered prior to the shock

It is seen in the table that many patients in group 1 underwent repeated conversions The ratio of the number of conversions to the number of patients is 1 5 The corresponding ratio in group 3 is 2 2 In other words there were more reconversions in the latter group Thus in spite of the fact that late recurrence of fibrillation is much more common in group 3 (i e group 3 represents a worse cardiac state than groups 1 and 2) the immediate post conversional complications discussed do not appear

The results should be considered in the light of this fact which further supports the assumption of a relationship between quinidine prior to the shock and an increased occurrence of post conversion arrhythmia

DISCUSSION

The procedure of discontinuing digitalis therapy before the DC shock has been well supported by clinical reports as well as by animal studies (10 11) Lown tested the energy levels necessary to start a ventricular tachycardia or fibrillation in a certain number of dogs (10) After having titrated the digitalis toxicity level for these dogs he repeated the experiment. He found a very marked decrease of the energy necessary to give the dogs ventricular tachycardia or fibrillation

Table I *Number of post conversion ventricular arrhythmias during different drug schedules at the time of countershock*

Drug regimen prior to shock	Time	No of pats.	No of conversions	Ventricular tachycardia and or fibrillation
1 Digitalis and quinidine	Aug. 1963 – Jan. 1966	160	234	4
2 Quinidine alone	Jan 1966 – Sept. 1966	29	38	4[b]
3 No therapy	Sept 1966 –	113	243	—

[a] One patient did not have quinidine
[b] One patient did not have quinidine This and one other patient were converted at the end of 1965 but did not receive digitalis during the last 2 days prior to the countershock.

when they were intoxicated by digitalis The reason for discontinuing digitalis prior to shock treatment would then be to minimize the number of undetected digitalis intoxications The number of such patients might easily be underestimated owing to the common use of diuretics with a potassium lowering effect potentiating digitalis The principle of discontinuing digitalis prior to a DC conversion is now generally accepted.

With regard to quinidine however the opinions differ (3 7 9 12 13) Rossi et al discuss the problem of quinidine administration at the time of conversion in a recent report (13) They conclude that quinidine prior to shock has several advantages for example to avoid early relapse into atrial fibrillation to make the procedure safer as regards complicating arrhythmias and finally a few patients are already converted by quinidine

The number of patients in their study was small There were 24 patients in the quinidine group and 23 in the control group

In the review of the results they graded the post conversion arrhythmias according to severity from zero to four Grade zero was no supra or ventricular ectopic beat and grade four ventricular bigeminy and ventricular tachycardia There were three patients in the control group belonging to grade four and one in the quinidine group No information is given as to which case or cases had ventricular tachycardia or ventricular bigeminy and nothing is said as to the seriousness of the arrhythmia Further the difference found is not significant (Fisher s *t* test)

Their results with regard to ventricular ectopic beats were in favor of the quinidine group but with regard to nodal rhythm in favor of the control group The numbers were small and the statistical analysis questionable however

There are some further objections to the study of Rossi et al In only 15 patients were the serum levels of quinidine determined and in this group the concentration ranged between 0 36–4 19 mg/l. The mean was 2 2 mg/l If these serum determinations are representative of the entire group the usually accepted maintenance level was not reached in their series Therefore their results are inconclusive as to the comparison between a group of patients on maintenance quinidine therapy at the time of conversion and a group without the therapy

Some other investigators who do not give quinidine prior to the shock have experienced no ventricular tachycardia or fibrillation (7 9) In a recent report by Radford and Evans no instance of these arrhythmias occurred in 34 patients immediately post conversion (12) Quinidine in a dose of 0 4 g had been given three hours before the shock, which should only result in a low serum level

Some interesting case studies by Castellanos et al also support the opinion expressed in the present paper (5)

If quinidine is not started until after the conversion there might be a few early relapses into atrial fibrillation These patients however still have a poor prognosis as regards maintenance of the sinus rhythm (4 12) The possibility of avoiding early relapses does not justify the risk of incurring post conversion arrhythmia through drugs administered prior to the shock treatment.

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THE RENAL METABOLISM OF CITRIC ACID IN RENAL TUBULAR ACIDOSIS

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Abstract The renal metabolism of citrate has been studied in a patient with renal tubular acidosis In accordance with previous studies, low citrate excretion was found during acidosis. The decreased clearance of citrate was related to an apparently high tubular reabsorption of citrate probably due to decreased formation and secretion of citrate from the tubules The amount of citrate metabolized in the kidneys was in the normal range Low normal citrate excretion was found after correction of the acidosis Correction of hypokalemia had no effect on citrate excretion

The kidney participates in the regulation of acid base balance by reabsorbing the filtered bicarbonate and by regenerating bicarbonate (7) The latter process depends on the formation of titratable acid and the secretion of ammonia The reabsorption and regeneration of bicarbonate are mediated by a common mechanism involving secretion of hydrogen ions in exchange for sodium both in the proximal and in the distal tubules The renal capacity for regeneration of bicarbonate is normally sufficient to restore the amount of bicarbonate decomposed in plasma by invasion of strong acids formed by metabolic processes in the organism If the regeneration of bicarbonate is less than the acid formed metabolic acidosis will ensue In such a condition the pH in urine will be low In renal tubular acidosis (RTA) the pH urine is high despite metabolic acidosis

Different pathogenetic factors for RTA have discussed (8 10) among others a deficiency activity of the enzyme carboanhydrase This ect will produce hyperchloremic acidosis which also characterizes RTA Blocking of carboanhydrase acetazolamide produces a decrease in bicar T_m and increased leakage of bicarbonate fferent from the pattern in RTA where bicar nate T_m is normal and the spillage of bicarbonate is diminutive (10) In addition normal activity of carboanhydrase in renal tissue has been found (12) A different distribution of carboanhydrase in the nephron might possibly cause a similar defect to that in RTA There is however no evidence to support this assumption

The disorder may be an inability to establish a high gradient of hydrogen ions between plasma and urine The reason for this defect in acidifying the urine is unknown The hypothesis of a back diffusion of hydrogen ions in the distal tubules due to permeability changes (7) is in our opinion too simple and bypasses the cellular mechanisms involved The urinary excretion of citrate is decreased in RTA (6 11) Milne et al (5) proposed that low excretion of citrate is related to intracellular acidosis Studies by Morrissey et al (6) lend support to this hypothesis After correction of the acidosis with bicarbonate a normal citrate excretion was seen by these authors In contrast Wrong (2) is of the opinion that decreased excretion of citrate is due to a deficiency of some enzyme essential for the normal functioning of the Krebs cycle This enzyme defect might be responsible both for the low citrate excretion and the inability to acidify the urine After correction of the acidosis he observed an increase of citrate excretion but not to normal levels

Inhibition of carboanhydrase produces reduced citrate excretion in urine depression of the renal metabolism of citrate while tubular reabsorption of citrate is normal or slightly depressed (1) Acetazolamide apparently also influences the aerobic renal metabolism with reduction of renal oxygen consumption (1) The present studies were undertaken to elucidate the renal metabolism of citric acid in RTA to give additional information on the pathogenesis of this disorder

Table I *Renal function studies and renal clearance metabolism and tubular reabsorption of citrate during metabolic acidosis of varying degree in a case of renal tubular acidosis*

	Renal vein catheterisations						Conventional clearance measurement		
	First examination			Second examination			Third examination		
Electrolyte and acid/base state pH		7 30			7 35			7 40	
pCO_2 (mm Hg) St bicarb	37		18	27		17	36		22
Na K Cl (mEq/l)	130	2 6	101	140	4 0	119	—	—	—
Citrate concentration in blood (mg/100 ml plasma)		2 32 (art blood)			3 06 (art blood)			4 08 (ven blood)	
Arterio venous difference (mg/100 ml plasma)		0 78			0 77				
Metabolized in kidney (mg/min)		3 00			2 90				
Filtered through glomeruli (mg/min)		1 77			2 57				
Excreted in urine (mg min)		0 10			0 32			1 00	
Inulin clearance (ml min)		76			84				
Renal plasma flow (ml/min)		389			417				
Citrate clearance (ml min)		4 48			10 58			24 51	
Tubular reabsorption of citrate (mg/min)		1 67			2 25				
Percentage of filtered citrate reabsorbed		95			87				

METHODS

The patient studied was a 17 year-old female who had suffered from RTA from the age of 4 months and had revealed nephrocalcinosis growth retardation systemic acidosis and hypokalemia

The firs examination was performed while the patient was acidotic Eight months later a second study was under taken after the patient had been treated more vigorously with bicarbonate but still was slightly acidotic The first two studies were done by renal vein catheterization In a third examination, after normal acid base balance was restored conventional clearance of citrate was determined

Citric acid was determined by the pentabrom acetone method The renal citrate utilisation was calculated in accordance with Herndon et al (4) Methods used for the determination of glomerular filtration rate (GFR) and renal plasma flow (RPF) were as previously described (1)

RESULTS

The results are presented in Table I and are aver age values from 2–3 periods The average arterial citrate level varied between 2 32 and 3 06 mg/100 ml which is in the normal range Clearance of citrate was significantly decreased during the first two studies After correction of the acidosis the citrate clearance was higher The amount of citrate utilized by the kidney was in the normal range while tubular reabsorption of citrate was signifi cantly above the upper normal limit

The renal metabolism measured as oxygen con sumption was normal in this patient indicating a normal metabolism of citric acid (1)

Normalisation of citrate excretion after correc tion of the acidosis supports the view that changes in intracellular pH are responsible for the altered urinary excretion of citrate Changes in intra cellular pH will in our opinion influence the formation and tubular secretion of citrate It has been suggested that potassium depletion might produce low citrate excretion Morrissey et al (6) found no increase in citrate excretion after potas sium administration in RTA with hypokalemia They observed increased citrate excretion to the lower limit of normal range after correction of the acidosis and hypokalemia with $KHCO_3$ These results indicate that low citrate excretion is not related to potassium depletion per se Our observa tions are in accordance with the results of Mor rissey et al During the second examination when the excretion of citrate was decreased and she was still acidotic the hypokalemia was corrected

Recently changes in the citrate content in renal tissue have been demonstrated in connection with different metabolic disturbances (9) Further exam ination of the renal metabolic defect responsible for decreased formation and tubular secretion of citrate in RTA necessitates renal tissue analysis of citrate content

The low citrate excretion may be an important

fa tor for the formation of nephrocalcinosis in our patient Nephrocalcinosis is also seen in other acidotic states for example chronic carboanhydrase inhibition (3) The citrate forms with Ca a ch late complex which is soluble but dissociates poorly and thus binds Ca ions which otherwise could precipitate from the urine

COMMENTS

Citrate clearance is normally 35 ml/min $\mp$ 14 (1) Citrate clearance in this patient varied from a sig nificantly decreased value in acidosis to a low value within the normal range after correction of the acidosis The reason for decreased citrate ex cretion was apparently increased tubular reabsorption of citrate About 70% of filtered citrate is normally reabsorbed (1) During acidosis the ubular reabsorption of citrate varied between 95 and 87% This apparently increased reabsorption of citrate may be the result of

(*a*) reduced tubular secretion of citrate
(*b*) increased oxidation of filtered citrate by tubu lar cells

Due to tubular secretion the calculated values of tubular reabsorption of filtered citrate in normal p rsons may be estimated too low

The amount of citrate metabolized was within the normal range and significantly higher than the amount reabsorbed in accordance with observa ions in normal persons (1) Thus we have ob- u an apparently increased reabsorption of citrate when the patient was acidotic while the amount metabolized was in the normal range This could indicate that formation and tubular secre tion of citrate are reduced in RTA

The pattern of tubular reabsorption and metabolism of citric acid in RTA is not in accordance the observations after inhibition of carbo- ,drase Acetazolamide produces a reduction f the amount of citrate metabolized while tubular reabsorption is in the normal range Our results ipport the opinion that carboanhydrase activity s normal in RTA

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RIGHT ATRIAL MYXOMA

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Abstract A review of the symptomatology of right atrial myxoma is given, based on 33 cases from the literature and on two cases studied by us

This diagnosis should be considered in any patient with isolated right heart failure without pulmonary congestion, pulmonary disease or pulmonary stenosis

A tricuspid murmur intermittent cardiac or cerebral symptoms or radiographic demonstration of non valvular intracardiac calcifications should increase the suspicion

Selective right atrial angiocardiography is the diagnostic method of choice The diagnosis cannot be excluded without this examination, which always should be performed in any patient with isolated tricuspid valvular disease

Primary tumors of the heart are rare Strauss and Merliss (30) found a rate of 17 primary tumors per million autopsies during the period 1938 to 1942 One half of the tumors reported by Prichard (25) were myxomas Seventy five per cent of the myxomas were located in the left atrium and about 25 % in the right atrium

Atrial myxoma is now a curable disease The first successful operation was performed in 1954 by Crafoord (11) under direct vision during cardiopulmonary bypass Since then several successful operations have been reported (26)

The diagnosis of right atrial myxoma is accordingly important It can be rendered probable by angiocardiography if only the disease is suspected Our purpose is thus to give a review of right atrial myxoma with emphasis on the symptomatology based on 33 cases previously published (1–10 12–24 26–29 31–33) and on two additional cases which we have recently encountered in this department

CASE REPORTS

Case I

A 51 year-old woman was admitted in February 1967 for ble mitral heart disease

There was no history of rheumatic fever At the age of 29 years she was admitted to a local hospital with symptoms of embolism of the left lung

Two years later at the age of 31 years she had another pulmonary embolus. Treatment with digitalis was started and thereafter continued

Ever since the last episode the patient had symptoms consisting of attacks of dyspnea at rest but also to some extent on exertion She had the feeling of being suffocated and drawn forward during these attacks but she had never fainted She had furthermore noticed that she was unable to lie on her left side because of dyspnea

For the last two years the symptoms progressed and she was admitted for diagnostic investigation

Physical examination revealed a slender woman in no distress There was no dyspnea at rest and no cyanosis Slight engorgement of the neck veins and venous pulsation with a big a wave was seen The liver was palpable 4 cm below the curvature There was no ascites or peripheral edema and no pulmonary stasis was found The blood pressure was 1 0/80 mm Hg

At auscultation of the heart a systolic murmur grade 2 (of 6) was heard at the apex and at the left lower part of the sternal border No diastolic murmur was noticed

The electrocardiogram showed sinus rhythm with a QRS axis of +110 degrees and peaked P waves in standard leads II and III

The roentgenogram of the chest showed moderate cardiomegaly with dense intracardiac calcifications (Figs 1 and 2)

Laboratory tests The hemoglobin was 14 6 g per 100 ml and the sedimentation rate 5 mm/h Electrophoresis of the serum proteins showed normal distribution A lung scanning with J 131 albumen was performed and showed reduced blood flow through the left lower lobe

Hemodynamic investigation At right heart catheterization the catheter could only with difficulty be passed through the tricuspid ostium. The pressure in the pulmonary artery was 17/5 mm Hg in the wedge position mean 5 mm Hg, in the right ventricle 17/0 mm Hg, and in the right atrium −1 mm Hg in mid-diastole The cardiac index was 1 4 l per m only

A selective angiocardiography and cineangiography with contrast injection into the right atrium was performed A large mobile mass a part of which was calcified was demonstrated in the right atrium.

An operation in extracorporeal circulation was performed and a yellowish tumor was completely removed from



Fig 1 Antero posterior roentgenogram of the chest of case 1 Note the calcifications of the right atrial myxoma



Fig 2 Lateral roentgenogram of the chest of the same patient as in Fig 1 Note the posteriorly situated calcifications

the right atrium The tumor was pedunculated being attached to the margins of fossa ovalis it filled the entire atrium and was hourglass-shaped The part facing the tricuspid ostium was calcified while the rest of the tumor was of brittle consistency Histological examination showed myxoma with calcification.

Postoperatively the patient developed arrhythmias mainly [consi]sting of intermittent atrio ventricular block, atrial flutter and atrial fibrillation One month after operation sinus rhythm was restored through a DC countershock At a follow up examination one year later she still had sinus rhythm however she still needed diuretic treatment because of moderate right heart failure and she had a systolic murmur grade 2 at the left lower sternal border—increasing during inspiration—and pulsation of the neck veins due to moderate tricuspid insufficiency

Case 2

A 53 year-old man was transferred from another hospital at the end of March 1967 for diagnostic investigation of possible constriction of the heart

He had never had rheumatic fever pleurisy or tuberculosis and he had previously been in good health

In August 1966 he was admitted for a few days to the local hospital because of an abnormally deep Q_{II} in the electrocardiogram which was taken incidently during a medical examination for insurance purposes His only symptom at that time had been one episode of dyspnea on climbing stairs one month previously Physical examination was normal except for obesity The roentgenogram of the chest was normal He was discharged with a diagnosis of old myocardial infarction

Four months later he developed increasing dyspnea on exertion, a persistent unproductive cough edema of the legs and swelling of the abdomen He was readmitted to the local hospital in March 1967 Hypoalbumenia was found in addition to elevated serum bilirubin and low prothrombin time The roentgenogram of the chest showed enlargement of the cardiac silhouette the electrocardiogram showed marked low voltage which was the main reason for the suspicion of constriction of the heart

Physical examination revealed an obese man in no acute distress There was slight dyspnea at rest and slight cyanosis of the ears, lips and nails Some engorgement of the neck veins was seen but no venous pulsation The liver was palpable about 10 cm below the curvature and there was marked ascites There was no peripheral edema. No pulmonary stasis could be detected The blood pressure was 130/80 mm Hg

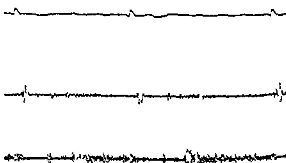


Fig 3 Phonocardiogram from the fourth left intercostal space recorded during inspiration in case 2 Note the long diastolic murmur

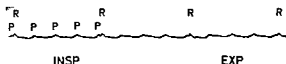


Fig 4 Phonocardiogram of case 2 during atrial flutter with 4 1 block. The diastolic murmur during inspiration is divided into short parts synchronous with the P waves

Auscultation of the heart The heart sounds were very faint, the second heart sound being barely audible In the fourth left intercostal space at the sternal border a peculiar murmur was heard it was a diastolic superficial scratching medium to high frequency murmur which was only present during inspiration (grade 2) being completely inaudible during held expiration. This was found when the patient had sinus rhythm nodal rhythm atrial fibrillation (Fig 3)

During part of his time in hospital the patient had atrial flutter with changing block, usually 4 1 block. In this rhythm the murmur was present during the whole inspiration as short parts interrupted by brief silent intervals The short parts of the murmur were synchronous with the P waves of the electrocardiogram Fig 4)

The electrocardiogram showed a markedly low voltage in all leads (Fig 5) The rhythm changed—as mentioned—between sinus rhythm with wandering pacemaker nodal rhythm atrial fibrillation and atrial flutter with usually 4 1 block

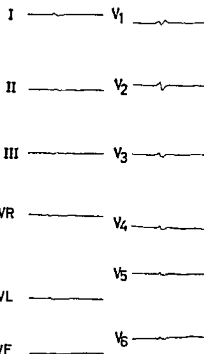


Fig 5 Electrocardiogram of our case 2

The roentgenogram of the chest showed a diffusely enlarged heart.

Laboratory tests The hemoglobin was 14 3 g per 100



Fig 6 Selective angiocardiography with contrast injection into the right atrium of case 2 Anteroposterior (*a*) and lateral (*b*) projection

ml, the sedimentation rate 4 mm/1 hour the serum bilirubin 3.5 mg per 100 ml the thymol extinction test 2, the alkaline phosphatase 49 units per 100 ml (normal upper limit 38 units per 100 ml) the lactic acid dehydrogenase 34 units per ml (normal upper limit 24 units per ml) with the elevation being confined to isoenzyme no 1 (myocardial fraction) and the prothrombin percentage was 25 There was slight hypoalbuminemia (4 1 g per 100 ml) and hypergammaglobulinemia (2 0 g per 100 ml)

Hemodynamic investigation At right heart catheterization the pressure in the pulmonary artery was 21/14 mm Hg, in the "wedge" position mean 11 mm Hg, and in the inflow tract of the right ventricle 33/7 mm Hg On withdrawal of the catheter to the right atrium a pressure gradient over the tricuspid valve of 19 mm Hg was found the mid diastolic pressure in the right atrium being 26 mm The pressure tracings did not show the characteristic configuration of constriction of the heart The cardiac index was 1 4 l per m and the arterial oxygen saturation 89 At selective angiocardiography and cineangiography with contrast injection into the right atrium a mass was seen extending from the right atrium to the right ventricle impeding the flow through the tricuspid valve and through the outflow tract of the right ventricle (Fig 6) Intracardiac phonocardiography demonstrated a diastolic murmur in the inflow tract of the right ventricle

At open heart operation in extracorporeal circulation a right atrial tumor of the size of a tennis ball was removed The tumor had a short, broad stem to the atrial septum close to the foramen ovale and it extended towards the tricuspid valve which was normal The tumor was soft and friable The histological diagnosis was pseudomyxoma The patient recovered slowly

At follow up examination one year later the patient was asymptomatic with sinus rhythm, and the low voltage had disappeared

SYMPTOMATOLOGY

Our review of the symptomatology is based on the case histories of 33 patients which have been published previously with sufficient information for our purpose and on our two patients presented above The histological diagno is was established at autopsy in three cases and at operation in the remaining 32 cases

There were 15 males and 20 females The age distribution at the time of the onset of symptoms is seen in Fig 7 The youngest patient was 7 months old and the oldest 57 years old

The various symptoms and signs may be divided into four groups as seen in Table I

Of the *constantly present symptoms* right heart failure was seen in all but two patients It was obviously due to the mechanical obstruction caused by the tumor Dyspnea was also commonly seen possibly due to a low cardiac output secondary to

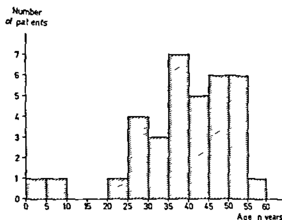


Fig 7 Age distribution of 35 patients with right atrial myxoma

the low venous return The low cardiac output might also explain the cyanosis and the signs of insufficient peripheral circulation

The *intermittent symptoms* which were seen in 22 patients were probably caused by a sudden increase in the obstruction to the blood flow through the tricuspid ostium e g provoked by the

Table I *Frequency of symptoms and signs in 35 patients with right atrial myxoma*

		No of pats		Initial in no of pats.
Constant				
Right heart failure		33		0
Dyspnea during exercise		27		19
Dyspnea at rest		12		1
Cyanosis		8		[illegible]
Insufficient peripheral circulation		6		[illegible]
Leg cramps	2			
Gangrene	1			
Raynaud s symptoms	1			
Thrombophlebitis	2			
Intermittent				
Dyspnea		11		1
In certain positions	7		0	
Nocturnal	2		0	
Unprovoked	2		2	
Cerebral symptoms		13		7
Syncope or dizziness	12		7	
Epileptic seizures	1		0	
Precordial or epigastric pain		13		
Cyanosis		9		0
Flushing		4		0
General				
Fever		7		2
Weight loss		7		1
Pigmentation		3		0
Pulmonary embolism		6		1

Table II *Auscultatory findings in 35 patients with right atrial myxoma*

	No of pats
Diastolic murmur in tricuspid area	24
Systolic murmur in tricuspid area	21
Systolic/diastolic murmur only at the apex	2
Gallop rhythm	11
Friction rub	6
Normal findings	2
Total	35

Table III *Electrocardiographic findings in 35 patients with right atrial myxoma*

	No of pats
Right atrial hypertrophy	19
Right axis deviation	8
Low voltage	18
Right bundle branch block	11
Sinus rhythm	29
Atrial fibrillation	4
Intermittent atrio ventricular block	3
Entirely normal	2
Total	35

assumption of a new position as the left lateral recumbent position The cerebral symptoms were often alarming and dominating The precordial and epigastric pain was often intense but otherwise uncharacteristic

Of the *general symptoms* which usually accompany tumors fever and weight loss were each found in one fifth of the cases

Pulmonary embolism occurred in six patients the embolus consisting of either tumor tissue or thrombotic tissue It was fatal in one case

The duration of the symptoms until correct diagnosis averaged 3 years ranging from five days to 36 years The duration was less than one year in 25% of the cases

Auscultation

As seen in Table II the auscultatory findings are mainly those of tricuspid valvular disease The findings are often transient and may vary with the position of the patient

The gallop rhythm was most likely due to tumor impaction as suggested by Sannerstedt et al (26) and the friction rub was probably secondary to affection of the pericardium as pericardial exudate was found in six cases

The diastolic atrial murmur which was interrupted and synchronous with the P waves (Fig 4) in our case 2 could easily be mistaken for a friction rub

Electrocardiogram

The findings are summarized in Table III In most cases a right sided heart disease was indicated by signs of right atrial hypertrophy (peaked P waves in II III aVF or right sided precordial leads) right axis deviation or right bundle branch block As many as half of the patients had unexplained low voltage (Fig 5)

Roentgenogram of the chest

A roentgenogram of the chest was obtained in all patients but one In 22 cases cardiomegaly was noted A prominent right heart border was seen in 18 cases In five patients the heart was normal

In four patients calcifications within the cardiac silhouette were noticed Pleural exudate was demonstrated in four cases Pulmonary congestion was not noted in any case

Laboratory tests

These were uncharacteristic in some cases consisting of anemia leucocytosis and elevated sedimentation rate

Right heart catheterization

Heart catheterization was performed in 27 patients In all but three patients a diastolic pressure gradient was measured across the tricuspid valve In two patients a pressure gradient was found over the pulmonary valve and over the outflow tract of the right ventricle respectively One patient had pulmonary hypertension due to recurrent tumor embolism

Cardiac output was measured at rest in 13 patients Seven patients had either a cardiac output lower than 3 5 l per min or a cardiac index lower than 2 1 per square meter per min

Angiocardiography

Selective angiocardiography with contrast injection into the right atrium was performed in 25 cases without complications In all cases the tumor was demonstrated

Table IV *Initial clinical diagnosis of 35 patients with right atrial myxoma*

		No of pats
Pericarditis		8
Unspe ific chronic	5	
Tuberculous chronic	2	
Acute	1	
Mitral stenosis		4
Tricuspid stenosis		4
Rheumatic fever		2
Ebst in s disease		2
Myocardial infarction		2
Carcinoid tumor		2
Myo arditis		1
Bacterial endocarditis (subacute)		1
Peripheral arterial insufficiency lower extr		1
Polycythemia vera		1
Addison s disease		1
Rheumatoid disease (arthritis)		1
Functional symptoms		1
No information		4
	Total	35

Prognosis and treatment

Three patients died without attempts at surgery the cause being heart failure in two cases and pulmonary embolism in one case In the remaining 32 cases surgery was performed

Eight cases were operated on without extracor real circulation and four died Twenty four cases were operated on in extracorporeal circula tion with 16 survivors The usual finding at opera tion was a pedunculated tumor by which the inter mittent symptoms could be explained Tricuspid insufficiency following operation was reported in five cases

DISCUSSION

The clinical diagnosis may be difficult as seen from the listing of the initial diagnosis in the 35 patients in Table IV

As soon as a myxoma has reached a certain size it will give rise to symptoms of which right heart failure is dominant In the presented material the lack of pulmonary congestion on the roentgeno gram and the majority of the electrocardiographic findings clearly pointed to the right side of the heart as site of the disease

Isolated right heart failure is found in pulmonary diseases pulmonary stenosis disease of the right ventricle constrictive pericarditis tricuspid valve disease and tumor of the right atrium The first possibility is easily ruled out Auscultation will in the ma ority of cases reveal a tricuspid murmur which in combination with a history of intermittent cardiac or cerebral symptoms should arouse the suspicion of myxoma Non valvular calcifications inside the cardiac silhouette point to the same possibility

Furthermore right heart catheterization which would be indicated in cases with isolated ri ht heart failure will usually show an isolated tricuspid stenosis and exclude constrictive pericarditis pul monary valve disease and disease of the right ventricl

As isolated tricuspid stenosis is extremely rare angiocardiography should be performed in all cases of apparent i olated tricuspid stenosis as well as in cases of isolated right heart failure in which the heart catheterization fails to give a definite diagnosis

Selective right atrial angiocardiography will demonstrate the tumor No complication to this examination in cases with right atrial myxomas has been reported

Removal of the tumor by open heart surgery must be performed

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THE ADRENOCORTICOTROPIC EFFECT OF D SERINE^{1} NORLEUCINE^{4} VALINAMIDE ^{25} β 1 25 CORTICOTROPIN (DW 75)

A Comparison with Genuine ACTH

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Abstract A comparison has been made in 15 healthy ndividuals between the adrenocorticotropic effect of a ynthetic pentacosapeptide (DW 75) and natural ACTH The synthetic preparation was found to be six times more potent than the genuine preparation and to have a slightly onger lasting effect.

atural adrenocorticotropic hormone obtained rom various species consists of a single un ranched sequence of 39 amino acid residues pecies variations occur but are confined to the mino acids that occupy positions 25 through 33 e active portion of the molecule (positions 1 hrough 24) is identical in all corticotropins inves igated Thus only a portion of the ACTH poly eptide chain starting from the amino-terminal nd is necessary for the full biological potency of ACTH

In 1961 Kappeler and Schwyzer (7) synthesized a tetracosapeptide containing 24 amino acids cor esponding to the first 24 amino acids of the ge uine ACTH This preparation has been extensiv ly studied and found to have a biological effect dentical with the natural hormone The activity f the tetracosapeptide equals 106 IU/mg whereas he activity of the natural corticotropin equals 15 IU/mg

In 1966 Boissonas et al (1) succeeded in syn hesizing a pentacosapeptide more resistant than he genuine hormone and its active synthetic ana ogue so far prepared It contains D-serine at the terminal and valinamide at the carboxyl termi al the methionine residue in position 4 is re laced by an isologous norleucine residue The nalogue is a D-serine^{1} norleucine^{4} valinamide-^{25} 1 25-corticotropin and has been named DW 75 This pentacosapeptide was shown by Doepfner (4) to have an activity of 625 IU/mg thus being six times more potent than genuine ACTH and its 1–24 analogue In man DW 75 has been found very active But as only preliminary studies have been made to compare the adrenocorticotropic effect of DW 75 and genuine ACTH a study of the effect in a larger series of healthy individuals was performed

MATERIAL AND METHODS

Fifteen healthy individuals aged 20–58 years were exam ined They were doctors nurses and laboratory tech nicians working at the University Clinic Rikshospitalet, Oslo In order to eliminate the endogenous secretion of ACTH, all individuals were given dexamethasone orally the first five individuals 0 5 mg the others 2 mg 8 h before the test and at the time of the DW 75 or ACTH injection. DW 75 (supplied by Sandoz Ltd. Basel Switzer land) and ACTH (Corticotropin Nyco) were given slow ly (1 min) by intravenous injection. Blood was taken for plasma 17 hydroxycorticosteroid (17 OHCS) estimation in heparinized tubes immediately before the injection and after 1 2 4 and 6 h The blood samples were spun in the centrifuge for separation of plasma and blood cells and the plasma was frozen All plasmas from ind vidual tests were analyzed simultaneously

Every individual was examined four times being given 2 injections of 5 and 10 IU of DW 75 and 5 and 10 IU of ACTH The interval between the tests in the same individual was at least four days

Plasma 17 OHCS concentration was determined by a modification of the Eik Nes (5) method as described by Brinck Johnsen and Solem ( )

No side effects were observed in any individual In four individuals redness of the face was observed on the day following the test day probably due to an ACTH effect. In this respect there was no difference between DW 75 and genuine ACTH

Table I *Mean increment of plasma 17 OHCS concentration (μg/100 ml) in 15 dexamethasone blocked healthy individuals 1 2 4 and 6 hours after i v injection of DW 75 and ACTH respectively*

	1 h	2 h	4 h	6 h
DW 75 5 IU	12 6	15 6	10 5	2 6
ACTH 5 IU	15 3	12 8	6 7	0 8
DW 75 10 IU	11 6	16 3	10 3	2 7
ACTH 10 IU	14 0	12 8	6 7	0 5
P	<0 10	<0 05	<0 01	0 05

Pooled values of DW 75 and ACTH respectively

RESULTS

Oral administration of dexamethasone 8 h before the injection of DW 75 or ACTH suppressed the endogenous production of cortisol satisfactorily At 0 h the concentration of plasma 17 OHCS for all tests averaged 1 7 μg/100 ml The increment of plasma 17 OHCS concentration above the value stated at 0 h is considered due to the effect of DW 75 or ACTH The changes in plasma 17 OHCS concentrations are shown in Table I

After 5 IU of DW 75 the plasma 17 OHCS concentration increased by 12 6 μg/100 ml at 1 h 15 6 μg/100 ml at 2 h At 4 h the increment was 10 5 μg/100 ml and at 6 h 2 6 μg/100 ml

An almost identical response was obtained for 10 IU of DW 75 At 1 h the plasma 17 OHCS was 11 6 μg/100 ml at 2 h 16 3 μg/100 ml thereafter 10 3 and 2 7 μg/100 ml

Five and ten IU of ACTH increased the plasma 17 OHCS concentrations in the same degree the levels being 15 3 and 14 0 μg/100 ml respectively Later on the values decreased slowly being almost identical with the 0 value at 6 h

Since identical effects were found for both dosages the results have been pooled for statistical evaluation Statistically the values obtained for DW 75 are higher than those for genuine ACTH 2 4 and 6 h after injection ($p < 0\ 05 < 0\ 01 < 0\ 05$ respectively) while there is no difference between the effects of the two preparations at 1 h Also there is no difference between the maximal increments above 0 h for the two preparations

DISCUSSION

In this study two important observations were made namely regarding the potency of the pentacosapeptide and the duration of its effect The maximal response of the two preparations was identical and since the activity of DW 75 equals 625 IU/mg or 10 IU/0 016 mg whereas 10 IU of genuine ACTH equals 0 1 mg it has been shown that DW 75 is six times more potent than genuine ACTH and its β^{1-4} analogue This is a confirmation of Doepfner s (4) study using the ascorbic acid depletion assay s c

Apparently DW 75 also has a longer lasting effect than genuine ACTH the plasma 17 OHCS values being higher for DW 75 than for the genuine preparation both at 2 4 and 6 h At 6 h after the injection of DW 75 the plasma 17 OHCS level is still 2 7 μg/100 ml whereas the plasma 17 OHCS level after ACTH has fallen to a very low level

This shows a more prolonged effect of DW 75 confirming the findings of Jenny et al (6)

This study gives no indication of the reason for the stronger and longer lasting effect of DW 75 It is well known that intravenously injected ACTH will disappear rapidly from the blood and blood disappearance half time value has been found to be only a few minutes According to Meakin and Nelson (8) this is due to a rapid enzymatic inactivation which can be prevented by heating the plasma to 60°C or adding cysteine However it also has been maintained (3) that intravenously injected ACTH is concentrated in the kidney and the liver and from these organs is given off little by little to act on the adrenals

The chemical configuration of DW 75 gives no support to either of these possibilities but a retardation of the enzymatic degradation is most likely The substitution of D-serine for L serine makes this preparation more resistant to degradation by aminopeptidases whereas valinamide instead of asparagine in position 25 increases the resistance to degradation by carboxypeptidases Furthermore it is well known that inactivation of the molecule results from the oxidation of methionine to methioninesulphoxide However in DW 75 this is prevented by substituting methionine by its isologue norleucine In this way a very potent preparation has been synthesized possessing greater stability in the plasma and the tissues

Most likely DW 75 possesses also another advantage as a synthetic polypeptide it has no tendency to cause allergic reactions No such reactions were observed in this study

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Table I *Muscle glycogen in chronic renal failure*

Case	Sex	Age	Diagnosis[a]	Diet[b]	Plasma		Glycogen	
					Creatinine (mg/100 ml)	Urea N (mg/100 ml)	(g/100 g wet weight)	(g/100 g glycogen and fat free solids)
G B	♀	38	CP	PR	14 6	118	0 83	4 04
K L	♀	31	CG		14 3	150	1 19	6 31
B L	♀	36	CP	I R	14 0	174	1 46	7 28
D A	♀	67	CP	PR	1? 3	130	1 05	5 42
K F	♀	60	CP	PR	[illegible] 4	160	2 08	11 4
T L	♂	61	CP	PR	10 0	120	1 16	5 82
M S	♀	40	NS	PR	11 7	90	1 22	6 31
B D	♂	32	NC	PR	13 2	97	1 30	6 13
Å T	♂	48	PC		16 4	147	1 58	7 91
A J	♀	53	PK		12 5	100	1 14	5 93
B B	♂	51	PK	PR	21 0	190	1 20	6 12
Å B	♂	4[illegible]	NS		10 2	85	1 34	6 89
L A	♂	18	CG	PR	15 2	105	2 14	1 [illegible] 2
B S	♂	18	CG		10 7	101	1 76	8 59
L I	♂	20	PC		16 0	97	1 42	6 86
B G	o	17	CG	PR	16 2	140	1 27	6 88
S T	♀	45	PK		11 4	144	0 83	4 [illegible]6
B S	o	50	PC	PR	18 1	111	1 [illegible]0	6 29
P M	o	59	CG		18 8		1 25	6 00
R H	♂	43	NS		15 8		1 28	6 5[illegible]
J S	♂	36	CG		25 2		1 18	5 5[illegible]

[a] CP = chronic pyelonephritis CG = chronic glomerulonephritis NS = nephrosclerosis PK = polycystic kidney disease
[b] PR = protein restriction

Acute renal failure (12 patients) See Table II

This group was heterogeneous with respect both to etiology and duration of renal failure In seven cases the acute renal failure was a complication of surgery Although during or after operation all the patients had a fall in blood pressure and other signs of shock all except K W had normal or raised blood pressure at the time of investigation Prior thereto case S N had undergone peritoneal dialysis for two days and had in addition received a large amount of 30 glucose i v

Of the other patients two had had a fall in blood pressure in connexion with the acute onset (S F and B L) but had normal blood pressure when the examination was made After five days anuria ca e S F had undergone peritoneal dialysis for two days and case B L was investigated in the course of peritoneal dialysis which had been started two days before

Methods

Muscle tissue was obtained from the quadriceps femoris muscle by means of needle biopsy (3) The trichloroacetic acid extractable glycogen was determined in duplicate muscle specimens according to Hultman (15) The neutral fat and water content were determined in separate muscle samples Since many of the uremic patients had an increased water content of the muscle and several also an increased content of neutral fat, the glycogen values were referred to glycogen and fat free solids as well as to wet weight

RESULTS

1 *Chronic renal failure* (see Table I)

All patients had normal muscle glycogen values with the exception of cases G B and S T who had slightly decreased values In a comparison with both normal healthy subjects and with hospitalized patients without renal failure or diabetes mellitus no significant difference was found between the mean values (Table III)

2 *Acute renal failure* (see Table II)

All but two patients had decreased muscle glycogen values Particularly in the group of postoperative renal failure most patients had extremely low values The only patients in this group who had normal values (S N and S F) had undegone peritoneal dialysis and hemodialysis respectively a short time before Case B L is of special interest When the first biopsy was performed diuresis had started and her serum creatinine had started to fall Her muscle glycogen value was extremely low During the following weeks she ate a normal hospital diet without

Table II *Muscle glycogen in acute renal failure*

Case	Sex	Age	Diagnosis	Days of		Plasma		Glycogen	
				oliguria	diuresis	Creatinine (mg/100 ml)	Urea N (mg/100 ml)	(g/100 g wet weight)	(g/100 g glycogen and fat free solids)
A *Postope ative renal failure*									
T J	o	44	Op aortic stenosis and incompetence	3		15 4		0 51	2 59
S B	o	44	Op aortic stenosis	5		18 0		0 31	1 51
S N	♀	54	Op ileus	9		9 0		1 12	5 54
K Å	♂	34	Cholecystectomy	9		15 6		0 51	2 24
K W	♀	70	Partial gastrectomy pancreatitis and peritonitis	8		10 7		0 14	0 67
E T	♂	39	Partial gastrectomy	5		17 2		0 .8	1 44
H B	♂	57	*Cholecystectomy* and partial gastrectomy	4		13 6		0 70	3 75
B *Other cases*									
A V	♀	72	Hemolytic crisis	8		9 8		0 72	
V L	♀	61	Fever hemolytic crisis	9		9 8		0 81	3 89
S F	♂	32	Carbon tetrachloride po soning	9		22 4		1 15	
A S	♀	37	Toxemia of pregnancy liver affection	1	2	4 2	74	0 55	3 42
B L	♀	59	Gastroenteritis endotoxin shock	3	1	6 8		0 27	1 37
					7	1 1		(0 66)	(3 34)
					17	1 2		(2 38)	(13 37)

restriction of protein or calories One week later the muscle glycogen had not been normalized When a determination was made after a further 10 days the muscle glycogen value was high

Table III *Muscle gl)cogen in control subjects and in patients with renal failure*

	n	Glycogen (g/100 g wet weight)	Mean and range (g 100 g glycogen and fat free sol ds)
subjects	.28	1 39 *(0 9 –2 49)*	6 69 *(4 33–12 66)*
·Iospital zed non urem and non-diabeti pat ents	16	1 40 (1 01–2 12)	
˜hron c renal failure	21	1 31 (0 83– 08)	6 93 (4 04–12 2)
Acute renal failure			
A Postoperative	7	0 51 (0 14–1 12)	2 53 (0 67 5 5)
B Other cases	5	0 70 (0 27–1 15)	. 89 (*n* = 3) (1 35 3 74)

DISCUSSION

1 *Chronic renal failure*

It is known that in man the basal metabolism of the skeletal muscle is maintained chiefly by fat combustion (2) whereas the muscles carbohydrate store is mainly utilized in muscle work (6 16) In diabetes mellitus there is no decrease in the ability to utilize muscle glycogen in muscle work (per sonal unpublished observations) but on the other hand the resynthesis of muscle glycogen is im paired due to insulin deficiency or a decreased sensitivity to insulin In juvenile diabetics we pre viously found (7) greatly reduced muscle glycogen values (mean 0 49 g/100 g wet weight) Adults with mild diabete *not requiring insulin also had* significantly lower values (mean 1 11 range 0 77–1 56 g/100 g) than non-diabetic hospitalized pa tients and normal subjects (28)

Some of the patients in this study who were on a low protein diet presumably had a relatively high carbohydrate intake On the other hand several of them were greatly affected clinically by uremia with anorexia and vomiting during the

period before the glycogen determination The effect of both fasting and a high-carbohydrate diet on the muscle glycogen in normal experimental subjects indicates that these diets have only an inappreciable effect unless heavy muscular work is performed before and during the examination (17) It is actually noteworthy that practically all these severely ill patients had completely normal muscle glycogen values

The fact that we found entirely normal muscle glycogen values in so many chronically uremic patients argues against the decreased sensitivity to insulin described in uremia involving the skeletal muscle to any great degree Campanacci et al (9) recently reported low muscle glycogen values in patients with chronic uremia which they interpreted as an expression of the same disturbance as causes decreased glucose tolerance Their results are not in agreement with those presented here Important differences however exist between the two series Thus most of the patients of Campanacci et al had been on extremely protein poor diet for a varying time before the determinations This may have contributed to the reduced glycogen values since lengthy protein deficiency is known to be able to affect the carbohydrate metabolism in man (22) A decreased glucose tolerance also s in dogs given a low protein diet (13) oreover it is possible that the biopsy specimens of Campanacci et al had a relatively high content of connective tissue in relation to muscle cells since in many cases they noted extremely low values for alkali soluble protein nitrogen in relation to fat free dry weight

2 *Acute renal failure*

The patients with acute uremia form a much more heterogenous group than those with chronic uremia In all but two patients who had undergone dialysis the muscle glycogen content was low Low values were recorded particularly in patients whose renal failure had developed as a complication of operative trauma

It is known that the glycogen content of the skeletal muscle falls considerably after an abdominal operation (5) We have also observed that on the fourth to fifth day after operation the patients have a decreased synthesis of glycogen after a standardized glucose infusion as compared to normal experimental subjects (unpublished observations) The postoperative stage has been described as a pseudo-diabetic state (23) In addition shock is known to lead to a rapid breakdown of muscle glycogen in experimental aimals (20) The extremely low muscle glycogen values which we found in postoperative patients with renal failure may therefore have arisen as a sequela of the operative trauma with resulting shock, i e the same factors as led to the patients renal damage No definite conclusions can be drawn from the type of case material with acute anuria in our study about whether the acute uremic intoxication as such had any effect on the muscle glycogen or whether the reduced muscle glycogen values constitute an unspecific component of these patients catabolic state

It is known that potassium and glycogen accumulate together in liver and muscle (4 11) approximately in proportion 0 5 mEq K per g glycogen A rapid breakdown of muscle glycogen, as occurs in acute renal failure will liberate considerable amounts of potassium from the cells and thus contribute to the development of hypokalemia

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Acta med scand Vol 186 pp 183–185 1969

PERMANENT ECTOPIC SUPRAVENTRICULAR TACHYCARDIA TREATED WITH AN ADRENERGIC β RECEPTOR ANTAGONIST

A Case Report

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Abstract A case of permanent ectopic supraventricular tachycardia followed for 11 years, the last two years on propranolol treatment is reported Untreated the heart rate varied spontaneously between 150 and 210 per min Quinidine procainamide Valsalva s maneuver and carotid massage had no effect. Acetylcholine intravenously and electroshock converted to sinus rhythm which however persisted only for a few seconds Digitalis treatment had no effect on the atrial rate but caused a varying degree of A V block (grade II–III) When the patient was treated with propranolol the ectopic focus was retarded until the sinus node could compete However when the treatment was discontinued there was a gradual acceleration of the firing of the ectopic focus which again became the leading pacemaker This indicates that the sinus node was less sensitive to adrenergic β receptor blockade than the ectopic focus This fact and the chronicity of the tachycardia are in accordance with a supposed hypersensitivity of the ectopic focus to sympathetic stimulation

A case of long lasting ectopic supraventricular tachycardia was first reported by Wilson and Herrman (4) in 1923 In 1954 Shachnow et al (3) were able to find only 13 reported cases of more than 2 months duration Since then another eight cases mostly children have been added to literature (1)

In the following a case of such long lasting atrial tachycardia is reported in which with an adrenergic β receptor antagonist (Inderal®) suppressed the tachycardia

CASE REPORT

The patient, a 30-year-old woman was first admitted to Ullevaal Hospital Department VIII in 1957 because of asthenia and tachycardia She had never been hospitalized before In her family no case of heart disease was known In the patient s childhood her mother had observed rapid huge pulsations in the carotids and shortness of breath on slight exertion, but a doctor was never consulted The patient felt relatively well however until the age of 30 when, during an attack of gastroenteritis, she became weak and dyspneic. The doctor called for found a tachycardia and gave her digitalis. As she did not improve she was referred to our hospital

Physical examination (16 9 1957) revealed a somewhat nervous woman but otherwise with a healthy appearance The heart rate was 150 per min and the blood pressure 115/85 mm Hg. The heart sounds were normal without murmurs and the relative heart volume was also normal (400 ml per square meter body surface) She had no signs of hyperthyreosis and the thyroid function tests were normal ECG from the digitalized patient (Fig 1) showed a supraventricular tachycardia with partial A V block (grade II–III) Quinidine procainamide Valsalva s maneuver and carotid massage did not convert the arrhythmia The patient was given 0 10 mg acetylcholine intravenously and a slower rhythm (rate 65 per min) with normal P waves (probably sinus rhythm) was seen but only for a few seconds The patient was discharged with digitoxin 0 10 mg per day and she still had an atrial rate varying between 150 and 200 per min but with A V block grade II and a ventricular rate varying between 80 and 150 per min After some weeks of rest she was able to return to her office work. In the following years the situation was unchanged In 1965 she complained of some dyspnea, but she had no signs of congestive heart failure In 1966 she felt tired, dyspneic and could barely manage light office work but a re nvestigation did not reveal any signs of heart disease except the atrial tachycardia Electroconversion was now tried but after a few sinus beats the atrial tachycardia continued

The digitalized patient was then given propranolol (Inderal®) 10 mg intravenously (Fig) and the atrial rate was reduced from 16_ to 1 6 per min The slower atrial rate led to a 1 1 A V conduction with prolonged PQ interval and the ventricular rate therefore increased from 100 to 1 6 per min All treatment was then stopped for a month without obvious clinical deterioration of the

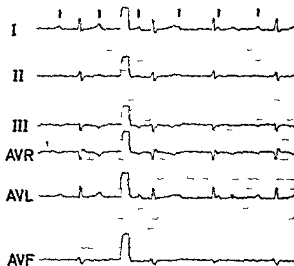
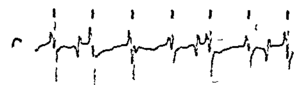


Fig 1 ECG 17.9.1957 of the digitalized patient. A supraventricular tachycardia with varying degree of A V block is seen. The atrial waves (rate 150 per min) are marked. Ventricular rate 95 per min. Paper speed 50 mm per sec.





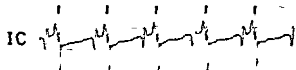




Fig 2 ECG 10.1.1966 of the digitalized patient showing the supraventricular tachycardia before (upper) and after (lower) 10 mg propranolol intravenously. After propranolol atrial retardation occurred but A V conduction improved to 1 : 1 and the ventricular rate therefore increased. IC = intracardiac ECG recorded from an electrode in the right atrium. The intracavitary atrial waves are marked. I = first standard lead. Paper speed 50 mm per sec.

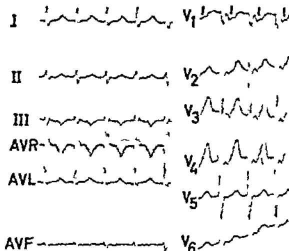


Fig 3 ECG 16.2.1966 of the resting patient after one month without drug therapy. The original tachycardia (rate 190 per min) with 1 : 1 conduction is seen. The atrial waves are marked in lead V. Paper speed 50 mm per sec. Lead aVR 20 mm = 1 mV.

patient. The ECG now showed a constant 1 : 1 A V conduction (Fig 3). The ectopic focus seemed to be located to the lower anterior portion of the right atrium, the atrial depolarization going dorsolaterally towards the left. The rate of firing of the ectopic focus changed spontaneously from 150 per min during sleep to 210 per min after slight exercise or with the patient standing. Propranolol 90 mg per day orally which was now given, reduced the atrial and ventricular rate to 80 per min and some normal P waves appeared (Fig 4). Propranolol 150 mg per day stabilized the sinus rhythm at the resting rate of 60 per min (Fig 5). On exercise some supraventricular

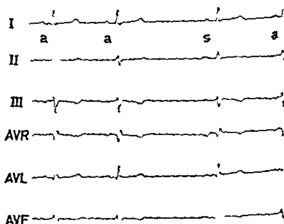


Fig 4 ECG 18 1966 during treatment with 90 mg propranolol per day. A retarded ectopic atrial focus (a) rate 80 per min and one sinus beat (s) are seen. Paper speed 50 mm per sec.

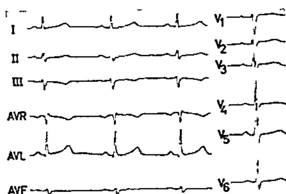


Fig 5 ECG 10.3 1966 during treatment with propranolol 1 0 mg per day Regular sinus rhythm with normal P waves is seen Lead aVL and V4 20 mm = 1 mV Paper speed 50 mm per sec

c beats still occurred, but the heart rate remained within normal limits From then on the patient has taken propranolol 120 mg per day and sinus rhythm has persisted. During the first week on propranolol she felt a bit drowsy and nervous because she "could not feel the heart beat in her chest" Later when she noticed the slower and regular pulse and her dyspnea improved she started training in gymnastics and swimming

In November 1966 the propranolol treatment was stopped for 36 hours The ectopic atrial focus soon accelerated to 160 per min, the normal P waves disappeared and the patient s dyspnea and fatigue returned When propranolol treatment was started again, the normal heart rate was reestablished, as was her sense of well-being. Up to date (December 1968) she still feels well and has no complaints

DISCUSSION

Chronic supraventricular tachycardia may be divided into a sustained and a repetitive type (2 3) No certain difference in the long term prognosis between the two types has been documented (2)

In our patient the tachycardia is of the sustained type It probably started in childhood but was first recorded at the age of 30 She has been under ation since 1957 and until propranolol treatment was started in 1966 ectopic atrial tachycardia s constantly registered Acetylcholine intra ly (in 1957) and electroshock (in 1966) l conversion to sinus rhythm but it did persist for more than a few seconds Propra ol however suppressed the ectopic focus and ince March 1966 she has taken 120 mg per day l at every examination a regular sinus rhythm of about 60 per min has been registered However as soon as propranolol is discontinued the ectopic center immediately starts the tachycardia anew

In most of the reported cases the sustained ectopic supraventricular tachycardia has been susceptible to a variety of stimuli and procedures in which the sympathetic and parasympathetic tonus seems to play a part It was therefore not surprising that the tachycardia was retarded by adrenergic β receptor blockade but unlike the β receptor blockade in ordinary paroxystic atrial tachycardia in which a conversion to sinus rhythm usually takes place the adrenergic β receptor blocking drug in this case of chronic supraventricular tachycardia caused a retardation of the ectopic focus an a competition between the sinus node and the ectopic focus occurred When 90 mg per day of propranolol was given the retarded ectopic focus was the leading pacemaker and the sinus node just captured a few beats When 120 mg per day was given the sinus node was the main pacemaker but the ectopic focus could still capture a single beat now and again This means that the tachycardia was never converted to sinus rhythm the ectopic focus was only retarded to a rate where the sinus node could compete indicating that the sinus node in this case is less sensitive to adrenergic β receptor blockade than the ectopic atrial focus This fact and the chronicity of the tachycardia are in accordance with a supposed hypersensitivity of the ectopic focus to sympathetic stimulation

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Acta med scand Vol 186 pp 187–191 1969

HEMODYNAMIC EFFECTS OF PROPRANOLOL (INDERAL®) AND H 56/28 (APTIN®) IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

A Comparative Study

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Abstract The hemodynamic effects of propranolol (Inderal®) and H 56/28 (Aptin®) were compared in a study of 17 patients in the acute stage of myocardial infarction. Eight patients received 5 mg propranolol i v (group I) and the remainder 5 mg H 56/28 (group A) Cardiac index (CI) heart rate (HR) and stroke index (SI) were significantly reduced after both drugs The difference between mean CI and SI reductions in the two groups was not significant ($p > 0.05$) while the difference between mean HR reductions was almost significant ($0.02 < p < 0.05$) Total peripheral resistance index (TPRI) circulation time (CT) and right atrial mean blood pressure (RAMP) increased significantly in both groups The difference between mean TPRI CT and RAMP increases in the two groups was not significant for TPRI ($p > 0.05$) but almost significant for CT and RAMP ($0.01 < p < 0.05$) Arterial systolic blood pressure (SBP) fell almost significantly ($0.02 < p < 0.05$) in group I while no change occurred in group A. Arterial diastolic and mean blood pressures did not change in either group

It is concluded that propranolol and H 56/28 have about equal effects on most of the measured parameters in patients with strong sympathetic drive The intrinsic sympathetic stimulating action of H 56/28 is not sufficient to compensate for the β adrenergic receptor blocking effect of the drug in this situation The two drugs should therefore both be considered dangerous in patients in whom augmented sympathetic drive is necessary for the [illegible] of adequate cardiac contractility and rate [illegible]he tendency to bradycardia, prolonged circulation time [illegible] hypotension may be slightly less after H 56/28 than [af]ter propranolol injected intravenously in the same doses

[β] adrenergic receptor blocking drugs have [pr]oved their usefulness in the treatment of certain [card]iac arrhythmias (10 13 14 16 18 19) in [angina] pectoris (1 4 6 7 11) and in some other [conditions] in which normal or augmented adren[ergi]c tonus plays a part in the pathogenesis For [re]view see Epstein and Braunwald (2)

The therapeutic and hemodynamic effects of these drugs mainly depend on their β adrenergic receptor blocking properties but their non specific myocardial depressive (9 21) and non specific anti arrhythmic properties (15 20) may also be of importance

The β adrenergic receptor blocking drugs in present use are either almost solely β adrenergic receptor antagonists or β adrenergic receptor antagonists with some intrinsic β sympathomimetic properties It still seems uncertain however whether the latter kind of drug in equipotent doses is less dangerous than the former in patients with impending heart failure in whom sufficient myocardial contractility and heart rate are dependent on augmented adrenergic tonus

When investigating the hemodynamic effects of new β adrenergic blocking drugs it seems important to test the drugs also in patients with relatively high sympathetic tonus This probably occurs in the acute stage of myocardial infarction or during physical exercise On the contrary in resting recumbent persons with no known cardiac affection the adrenergic activity is presumably small (12) Thus the hemodynamic consequences of β adrenergic receptor blockade under these conditions would be limited provided that the agent does not exert sympathomimetic action

In the present study of patients in the acute stage of myocardial infarction an attempt was made to compare the hemodynamic effects of propranolol (Inderal®) which is a quite specific β adrenergic receptor antagonist with those of H 56/28 (Aptin®) which besides being a potent β adrenergic receptor blocking drug also has some β sympathomimetic properties

by propranolol than by H 56/28 The systolic blood pressure fell almost significantly after propranolol, while no change was seen after H 56/28

Propranolol and H 56/28 both have important and about equal effects on most of the measured parameters in patients with strong sympathetic drive The intrinsic sympathetic stimulating action of H 56/28 is not sufficient to compensate for the β adrenergic receptor blocking effect of the drug in this situation The two drugs should therefore both be considered dangerous in patients in whom augmented sympathetic drive is necessary for the maintenance of adequate cardiac contractility and rate The tendency to bradycardia prolonged circulation time rise of right atrial pressure and hypotension may be slightly less after H 56/28 than after propranolol injected intravenously in the same doses

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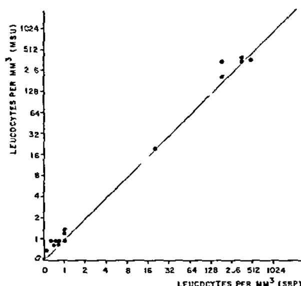


Fig 1 Comparison of leucocyte counts in mid-stream urine (MSU) and in urine aspirated by suprapubic bladder puncture (SBP)

coverslip (4) only clearly visible cells were counted (depth of the field approximately 0 025 mm)

Renal concentration ability and the leucocyte excretion rate have been estimated simultaneously At 4 p.m 5 I U of Pitressin® Tannate in Oil (Parke Davis & Co) were given subcutaneously (2) The exact time of the first ding on the next morning was noted During the next ding a midstream specimen was collected Estimation of diureses per hour was based on the total volume of urine of the second voiding and the time interval between the first and the second voidings. A specimen from each of these voidings was stored at −18 C for later determination of the osmolality

The mid stream specimen from the second voiding was used for another urine culture and leucocyte count. After careful stirring of the specimen, one drop of Sternheimer Malbin s or Prescott s stain was added to one ml of the urine Leucocytes were counted in a Fuchs-Rosenthal counting chamber Two hundred, or all leucocytes in 3.2 mm were counted (5)

Disrupted or degenerated cells were disregarded The coefficient of variation in cell counting has been found to increase from 9 to 17 as the number of leucocytes decreases from 65 to 4 per mm The counting results were independent of the staining method employed.

The osmolality of the urine specimens was estimated at Medical Department B Rigshospitalet, Copenhagen, by determination of the freezing point depression with an Advanced Osmometer

RESULTS

In 25 cases the leucocytes in urine aspirated by suprapubic puncture and in mid stream specimens collected in connection with the puncture were counted Fig 1 shows good agreement between the leucocyte concentration in the bladder urine and in mid stream specimens

The number of leucocytes per mm³ of urine has been compared with the leucocyte excretion per hour The urine from 97% of 164 patients who excreted more than 400 000 leucocytes per hour contained more than ten leucocytes per mm³ Ten or fewer leucocytes per mm³ were found in 92% of 71 cases excreting fewer than 400 000 per hour

With the technique employed for microscopic examination of urinary sediment it has been demonstrated that all patients with three or more leucocytes per HPF excreted more than 400 000 leucocytes per hour (19) If there were fewer leucocytes per HPF pyuria could not however be ruled out

The results of microscopic examination of the urinary sediment of all patients referred to the clinic are shown in Table I Even though three or more leucocytes were found in 75% of the 219 patients with bacteriuria and in 38% of the 253 women without significant bacteriuria, the probability of bacteriuria increased with the number of leucocytes seen per HPF

In 144 women of 152 with bacteriuria the leucocyte excretion rate exceeded 400 000 per hour (Fig 2) The excretion rate was found to be of the same magnitude in bacteriuric patients regardless of the type of organism isolated from the urine None of the 42 convalescents with no history or sign of renal disease excreted more than 400 000 leucocytes per hour

Table I *Maximum number of leucocytes per HPF seen by microscopic examination of the sediment in urine specimens from 472 women with symptoms of urinary tract infection*

	Leucocytes per HPF				Unknown	Total
	0–2	3–5	6–20	>20		
No of patients without bacteriuria	158	32	37	22	4	53
No of patients with bacteriuria	54	31	65	68	1	19
All patients examined	212	63	102	90	5	47
Per cent with bacteriuria	25	49	64	76		

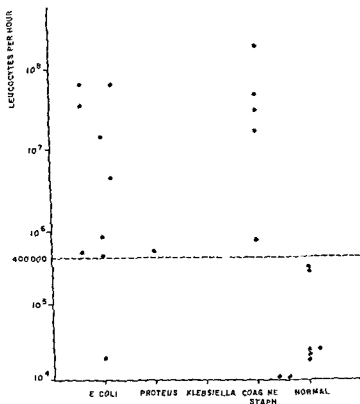


Fig Leucocyte excretion rate per hour in 42 normal subjects and in 15_ women with urinary tract infection shown in relation to the infecting organisms

Since February 1967 the maximal urine concentration ability (2) has been investigated in the bacteriuric patients In these patients impaired concentration ability is regarded as a sign of pyelonephritis (13 32) In 129 patients the leucocyte excretion as well as the concentration ability was determined It was found that the leucocyte excretion tends to increase as the concentration ability decreases (Fig 3) All but one of 26 patients who excreted fewer than 10^6 leucocytes per hour had a concentration ability of more than 750 mOsm/kg which is considered normal (2)

In four patients with uncomplicated urinary tract infection the leucocyte excretion rate was studied during treatment On the second day of treatment all the patients had sterile urine and the leucocyte excretion rate decreased rapidly within the first few days (Fig 4)

All the patients with bacteriuria were placed under continued observation In 46 cases of reinfection (44 patients) the last urine specimen examined before recurrence of bacteriuria was ob-

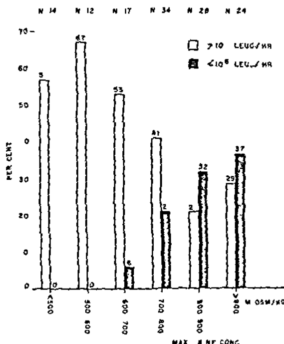


Fig 3 Leucocyte excretion in relation to the maximal urine concentration ability in 129 women with bacteriuria.

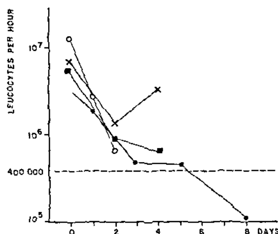


Fig 4 Leucocyte excretion rate during the first days after start of treatment

tained after a period of at least two months with sterile urine Table II shows the results of micro scopic examination of urinary sediment before and during the period of bacteriuria and the first weeks after the start of treatment Three or more leuco cytes per HPF were found in 15% of the speci mens examined before reinfection and in 78% of the specimens collected during the period of bac iuria After one month with sterile urine three more leucocytes per HPI were seen in 13% the specimens

DISCUSSION

The diagnosis of pyuria

Simple microscopic examination of urinary sedi ment has been found unsatisfactory for the detec tion of pyuria (16 19) The introduction by Ham burger et al (8) and Houghton and Pears (9) of simp e and reliable methods for quantitative deter mination of leucocyte excretion has made it possible to make a more exact diagnosis of pyuria

Quantitative methods require estimation of the leucocyte concentration using a counting chamber It has been shown that the coefficient of variation in counting increases as the number of cells per mm³ decreases (5 19 24 29) In order to reduce the error in counting several authors have con centrated the specimens by centrifugation and resuspended the deposit in one tenth of the origi nal volume It has been demonstrated that centri fugation and presumably also the resuspension are inadequate and the leucocyte concentration thus obtained will be too low (4 29)

Another problem is the differentiation of non squamous epithelial cells and leucocytes (27) The two types of cells are of nearly the same size but non squamous epithelial cells vary more in shape are often elongated and angular (15) Leucocytes are easier to recognize after staining with Stern heimer Malbin s stain (30) or after staining accord ing to Prescott and Brodie (26)

In the present study good correlation was found between the leucocyte concentration in urine aspi rated by suprapubic puncture and in mid stream specimens In a simular comparison of specimens from 12 puerperal women Roberts et al (28) found considerable deviations in some of the patients It is possible that the discrepancies have to some extent been due to the great risk of conta mination from vaginal discharge in these patients

In order that the diuresis may be determined within a short time the importance of complete emptying of the bladder is obvious To ensure this as far as possible without catheterization determination of the diuresis was based on two

Table II *Results of microscopic examination of urinary sediment in relation to 46 reinfections in 44 patients All these patients had a period of at least two months with sterile urine prior to the last urine examination before the reinfection*

	Before reinfection	Reinfection		After start of treatment		
		1st specimen	nd specimen	1 week	2 weeks	4 weeks
Max no leucocytes/HPF						
0-2	39	10	11	32	32	35
3-5	6	8	12	5	3	3
6-20	1	16	12	5	5	2
>20	0	12	10	0	1	0
No of specimens	46	46	45	42	41	40

consecutive voidings without the patients being instructed to urinate at a predetermined time

Healthy subjects have been found to excrete fewer than 400 000 leucocytes per hour (4 9 10 16 24) A slightly higher upper limit was found by Prescott (27) It has been found that women have a higher excretion rate than men (16 27) No correlation between age and excretion rate has been demonstrated (9)

Pears and Houghton (25) and Osborn and Smith (24) have found the rate of excretion to be rather constant in the individual patient while Montgomerie and North (23) and Prescott (27) have found extreme variation in some subjects Only minor variation between diurnal and nocturnal excretion rate has been demonstrated (6 27) Very little is known about the influence of urine flow on the leucocyte excretion (7 25)

Pyuria is defined as excretion of more than 400 000 leucocytes per hour The diagnosis of pyuria may be made by quantitative determination of leucocyte excretion However the urine of nearly all patients with pyuria contained more than 10 leucocytes per mm^3 while the presence of 10 or fewer leucocytes per mm^3 is seldom associated with pyuria It must be mentioned that these studies were performed on urine specimens collected during periods of low urine flow Comparison of the excretion rate and the number of leucocytes seen per HPF by microscopic examination of the urinary sediment has shown that three or more leucocytes per HPF indicate pyuria while fewer leucocytes per HPF do not exclude pyuria (16 19)

Pyuria in bacteriuria

As in other studies (12 31) it was found that ordinary microscopic examination of the urinary sediment revealed pyuria in only about seventy per cent of the patients with bacteriuria Among the women with urinary tract symptoms but without bacteriuria more than three leucocytes per HPF were found in 38% It must be considered that pyuria in some of these patients had been caused by chronic pyelonephritis with temporarily sterile urine or chronic glomerulonephritis (3) Others may have had urinary tract infection with fewer than 10 000 bacteria per ml in two consecutive specimens (20) or have spontaneously attained sterile urine before the examination Twelve of the patients had been treated with chemotherapeutics or antibiotics within the last week before the examination

Microscopic examination of urinary sediment also proved an unsatisfactory means of detecting reinfection

In patients with more than 10 bacteriuria per ml urine Fairley and Barreclough (3) found an excretion rate of more than 300 000 per hour in 93 of 117 patients examined Osborn and Smith (24) more than 200 000 per hour in all of 19 patients and Little (17) more than 200 000 per hour in 72% of 265 pregnant women In these studies leucocytes were counted after centrifugation of the specimens Mond et al (22) found more than 10 leucocytes per mm^3 uncentrifuged urine in all of 38 patients with bacteriuria

In the present study 144 (95%) of 152 women with bacteriuria excreted more than 400 000 leucocytes per hour Whether in the remaining eight patients the bacteriuria was present without invading or causing inflammationary changes in the tissue of the urinary tract, cannot be determined However the fact that signs of inflammation were found in 95 of the patients with bacteriuria supports the generally accepted view that bacteriuria indicates infection in the urinary tract (14)

The magnitude of leucocyte excretion was found to be independent of the type of bacteria isolated from the urine This strongly supports the view that coagulase negative staphylococci are pathogenic in the urinary tract (21)

Impaired urine concentration ability in bacteriuric patients must be regarded as a sign of pyelonephritis (11 32) The leucocyte excretion rate was found to be lower in patients with normal concentration ability i e over 750 mOsm/kg (2) Leucocyte excretion seems to be higher in patients with upper urinary tract infection than in patients with lower urinary tract infections On the other hand Hutt et al (10) have found that only six of 14 patients with acute or chronic pyelonephritis and "positive urine culture excreted more than 10^5 leucocytes per hour

It must be concluded that simple microscopic examination of urinary sediment is an unsatisfactory means of revealing pyuria If pyuria is defined as the excretion of more than 400 000 leucocytes per hour a few patients with bacteriuria may not have pyuria but this does not exclude the possibility of inflammation in the urinary tract For all practical purposes bacteriuria must be

regarded as an indication of urinary tract infection. As a consequence of this examination for pyuria is of lesser significance if bacteriuria has been demonstrated.

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PULMONARY HAEMOSIDEROSIS AND GLOMERULONEPHRITIS

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Abstract Three cases with the combination pulmonary haemosiderosis and glomerulonephritis (Goodpasture s syn drome) are described All the patients died A temporary improvement was seen in two cases with steroid treat ment In one of the patients the disease appeared in two distinct bouts In the first she had a localized lung process and microscopic haematuria. After lobectomy the state was normalized but one year later she had a rapid deterioration with pulmonary haemorrhages and glomer ulonephritis

The syndrome described in 1919 by Goodpasture (6) consists of acute glomerulonephritis and pul monary haemosiderosis In medical publications from Scandinavian countries cases have been re ported from Norway (14) Denmark (19) and Fin land (20) No case has as far as we know been reported from Sweden

Idiopathic pulmonary haemosiderosis as a diag nostic entity is known since the 19th century when it was described by Virchow (21) The first time the disease was diagnosed in vivo was by Waldenstrom (22 23) in 1940 Since then the number of cases published exceeds several hun dreds Goodpasture s syndrome (lung haemorrhage and glomerulonephritis lung purpura with nephri tis pulmonary haemosiderosis and nephritis haem orrhagic and interstitial pneumonitis with nephri tis the haemorrhagic pulmonary renal syndrome) is very vaguely defined and we think it is justified to describe three cases

CASE REPORTS

Case 1

L R a 28 year-old woman who fell ill Nov 1957 with acute tonsillit s She was treated for eight days with peni cill n After that she had se eral relapses of tonsillitis for a month or more Also signs of swollenness of the knees were noted The antistreptolysin titre (AST) was 1000 I U and fell after treatment to 210 I U There are no reports of urinary analysis on this occasion. During the winter of 1959 the patient had several attacks of sore throat and on Febr 9 1960 red blood cells were noted for the first time in the urinary sediment During this time the patient complained of increased nasal discharge She was then given penicillin but because of generalized pain and palmar itch the peni illin was stopped and chloramphenicol was instituted On Febr 29 she was a ain feverish and at the same time haemolytic streptococci type A 10 were isolated from her throat

First admission On Mar 1 1960 the patient was admitted to the Department of Internal Medicine She was pale and there was a notable sign of transparency in her face There were no signs of cardiac decompensation or jaundice Blood pressure 135 mm Hg systolic and 75 mm Hg diastolic The urinary sediment contained several red blood cells NPN was 45 mg% Hb 90 g ESR 110 mm haptoglobin 232 mg% and serum iron 32 μg%

On Mar 2 1960 she suddenly had a serious haemoptysis X ray showed in the middle part of both lungs rather symmetrical very widespread densities with diffuse borders against a normal upper and basal lung paren chyma In the densities an irregular mottling could be defined from smaller opacities of varying size The p ture corresponded well with that of recurrent pulmonary haemosiderosis During a fortnight there were period of im provement interchanging with short relapses On April 26 1960 the lung picture was definitely normal

The patient was dyspnoeic even at rest and had a pulse rate of 120/min On the tentati e diagnosis of pulmonary haemosiderosis steroid treatment (triamcinolon) was in stituted and the patient underwent immediate impro e ment On Mar 15 the serum creatinine was 3 16 mg % The steroid dose was at that time lowered to 8 mg triam cinolon day and the patient s cond t on deteriorated. As before the e were se eral red blood cells in the sediment. The Hb was 58 g and the ESR 105 mm The patient thus h d a new relapse of her pulmonary haemorrhage and the steroid dose was again increased to 16 mg/day On this t eatment she aga n improved a defir regre ion of the X ray f nd ngs in the

Second admission On Sept 4 1960 the patient was admitted to the Department of Infectious Diseases because of acute gastroenteritis The Hb was at that time 51 and there were a few red blood cells in the urinary sediment Serum electrophoresis showed a slight increase of alpha 2 globulins but was otherwise normal The patient was discharged improved

Third admission On Febr 5 1961 the patient was again admitted to the Department of Infectious Diseases after she had fallen ill with headache vomiting and a feeling of swollenness in her body At admission her blood pressure was 200 mm Hg systolic and 1 0 mm Hg diastolic The Hb was 45 the ESR 28 mm and the urinary sediment contained no red blood cells The serum creatinine was 7 7 mg An X ray of the chest showed minor relapses of the changes from April 1960

Because of the renal insufficiency steroid treatment was considered contraindicated On Febr 19 a tender gland was noted in the right submandibular space and penicillin treatment was started On Febr 22 the patient was transferred to the Department of Internal Medicine Here a harsh systolic murmur was heard all over the precordium The blood pressure was 180 mm Hg systolic and 120 mm Hg diastolic The urinary output was low There were also signs of pericarditis, pleuritis and exudative arthritis

On the chest film (bedside) enlargement of the heart and fluid in both pleural sinuses was seen but no parenchymal process of haemosiderosis type The patient was given Actocortin intravenously but without improvement

On Mar 1 she was transferred to the Haemodialysis Centre in Lund where a dialysis was performed on Mar The patient died after 5 hours of treatment

Pathological findings Terminally the patient was treated in a clinic for kidney disease at another hospital and was autopsied there According to the report small kidneys were found the right kidney weighed 80 g the left kidney 100 g The surfaces were brown red coarsely granulated with several small haemorrhages The cortex was slightly reduced

The right lung weighed 380 g the left lung 320 g they were heavy with increased density and from the cut surfaces especially in the upper lobes abundantly foamy pink-coloured fluid poured out

Microscopically most of the glomeruli were hyalinized with diffuse interstitial infiltration of lymphocytes and plasma cells Only a few intact glomeruli were seen and within these there were adherences between the capsule and the capillary tufts Also scattered crescent shaped epithelial proliferations were seen in the capsule There were no signs of vasculitis

In the lungs there were round lesions where the septa and alveolar walls were fibrotic containing a large number of iron pigment loaded macrophages There were also large irregular areas with fresh bleedings and several macrophages containing haemosiderin

Case 2

N B E an 8 year-old boy who had previously been healthy fell ill on Au 2 1961 with fatigue sore throat and a slight rise of temperature

First admission On Aug 14 dark-coloured urine was noted and the patient was admitted to the Pediatric Clinic at Malmo General Hospital There were several red blood cells in the urinary sediment and albuminuria The Hb was 49 red blood cells 2 4 mill ESR 60 mm, and serum iron 23 μg Blood pressure was 170 mm Hg systolic and 75 mm Hg diastolic The preliminary diagnosis was acute glomerulonephritis The patient complained however of cough and shortness of breath even after slight exercise During the following days the blood values fell and on Aug 25 the Hb was 31

X ray of the chest Aug 16 In both lungs rather dense widespread mostly perihilar and basal parenchymal infiltrates more on the right than on the left side The apical regions seemed to be free The infiltrates were rather diffuse peripherally and more patchy near the hilar regions They did not respect the borders between the cortex and the central parts of the lungs speaking against a uraemic cause The picture corresponded well with that of acute pulmonary haemosiderosis

The patient was treated with corticosteroids initially 30 mg of prednisolone daily The AST was 360 IU During the whole time there was haematuria with 0-30 red blood cells in the urinary sediment and also granular and hyaline casts The patient was discharged improved on Oct 25 1961

Second admission On July 5 196_ the patient was again admitted to the Pediatric Clinic because of abdominal pain, increasing fatigue and dark coloured urine X ray of the chest was at this time normal Haemolytic streptococci were found in the throat The AST was 600 IU The patient was for a short time treated by elimination of milk products (7) There was no improvement on this treatment Instead the patient was given corticosteroids, improved again and was discharged in a fairly good condition on Aug 10 196_ The Hb was then 53 and the serum electrophoresis showed a slight increase in alpha and gamma globulin

Third admission On Oct 10 1962 the patient's condition deteriorated He experienced shortness of breath and had several haemoptyses On X ray a typical relapse of an acute pulmonary haemosiderosis was seen

The patient was again treated with corticosteroids, starting with 30 mg prednisolone During this treatment there was definite regression of the lesions and the patient was discharged improved on Dec 19 The Hb was then 51 and serum iron 43 μg

Fourth admission On X ray examination of the chest on Jan 17 1963 there were signs of relapse Therefore the patient was again admitted to the Pediatric Clinic There were no catarrhal symptoms but the patient had vomited several times during the last day and was complaining of slight abdominal pain On this occasion too prednisolone treatment was started 30 mg daily During this treatment the patient improved He was discharged on continued steroid treatment

Fifth admission On April 15 1963 the patient was again admitted to the same clinic During the last day he had vomited quite a lot and these vomits had been blood

tinged In connexion with this his prednisolone dose was increased so that at admission it was 30 mg daily The patient now had manifest oedema and hypertension 165/130 mm Hg Because of this hypertension he was treated with alphamethyl-dopa

The Hæmodialysis Centre in Lund was consulted but there was no indication for haemodialysis at this time The patient s Hb was 77 On May 4 it had fallen to 46

In July 1963 the patient was on holiday in northern Sweden On his way home he was admitted to the Chil dren s Hospital in Sundsvall where he had some serious haemoptyses which in the course of a few days killed him

On admiss on to the Children s Hosp tal general malaise dyspnoea with rales over the lung bases and a typical Cushingoid picture was noted

Patholog cal find ngs At autopsy the organs had been placed in a small amount of formal n without previous d ssection and because of this the quality of the prep ara es was somewhat inferior

Microscopically large bleedings as well as lots of macro phages containing iron pigment were seen in the lungs The alveolar walls were somewhat thickened but there was only sl ght f brosis In many areas the alveoli con tained exudate polymorphonu lear leukocytes and fibrin

In the k dneys most of the glomeruli were hyalinized In some glomeruli, however capillaries were seen which showed a high degree of hyalinization of the wall and in some places there was a proliferation of the capsular ep thelium with crescent formation Many tubuli were destroyed to a high degree and replaced by fibrous tissue There were no signs of vascul tis

Case 3

M L a 50-year-old unmarried children s nurse who be cause of her work, was controlled once a year with fluoroscopy of her lungs The patient fell ill on May 22 1963 with fever Fluoroscopy of the lungs which was performed on May 27 showed rounded lesions basally on the right side The patient was then treated with penicill n because of fever and cough with no effect on the temperature and therefore the treatment was changed to tetracycline This treatment had no effect either on her fever The patient had on this occasion had no haemoptysis

F st admiss on The patient was admitted to the Depart ment of Lung Diseases Malmo General Hospital on June 14 1963 At this time she had signs of general malais and was distinctly pale There were no physical s gns from the lungs Because of a positi e Mantoux react on, treatment with PAS INH and streptomycin was instituted on June 17 The X ray changes remained un changed on the right side and therefore a bronchoscopy was performed on July 2 which showed a sl ght ir regularity in the mucous membrane of the right lower lobe No biopsy was taken

Bronchography right lung The bronchi of the upper and midd e lobes were normal In the lower l be th bronchi of the apical segments were somewhat dislocated in a caudal direction Some small ramifications showed small mucosal intraluminar irregularities There were no signs of a process starting on the bronchial system

Several cytological examinations of sputum showed no signs of malignant cells but in one specimen cell atypia was noted Furthermore a few macrophages containing haemosiderin were seen

Other examinations and laboratory tests X ray uro graphy normal Guinea pig inoculation test three times negative AST 125 I U CRP 20 mm Urinary sediment showed 10–12 red blood cells The Hb was 73% and the ESR 108 mm

Spirometry showed decreased function of the left lung and this finding was interpreted as a restrictive lung dis ease of moderate degree

On July 4 the patient was transferred to the Clinic of Thoracic Surgery where on July 10 a lobectomy of the lower lobe of the right lung was performed The lobectomy was without compl cation and the patient was discharged from the hospital on July 24 in good condition

Pathological findings Examination of the surgery speci men showed a rounded and fairly well defined tumour of 4 cm diameter in the right lower lobe The consistency was soft and the cut surface greyish wh te

Microscopically the normal alveolar structure was totally lost in the central parts and replaced by collagenous fibrous tissue containing several lymphocytes plasma cells and scattered eosinophilic polymorphonuclear leukocytes Furthermore irregular giant cells of foreign body type were seen but no epithelioid cell granuloma. No aniso tropic material was seen (Fig 1) The walls of some vessels were thickened and in a few places inflammatory cells were seen in the wall However there were no fibrinoid necroses and the picture was in the first place consistent with a secondary vascular engagement and not a primary vasculitis In the periphery of the lesion mark edly thickened alveolar septa were found, surrounding small cavit es The septa were mostly compact and rich in collagen but in some places especially in the periph ery they were loose and contained some fibroblasts In PAS (periodic acid-Sch ff) stainin no real basal mem branes were seen only faintly stainable slightly spl t-up material The elastic fibrils were of normal appearance Both in the alveoli and in the fibrous tissue a large num ber of haemosiderin-containing macrophages were found and in some places also groups of macrophages containin fat positive substan e The findings did not, however cor respond with those of lip d pneumonia Tubercle bacilli fungi or other paras tes were not present.

The lesion was interpreted as a chronic inflammatory process of unknown natu e

The patient v s ted the Clinic of Thoracic Surgery on several occasions and on May 5 1964 a microscop c haematuria was noted a f nd ng which had been noted lso at the exam nations in July 1963

S o d adn s on On Aug 0 1964 the patient fell ill w th high fe er general malaise so eness in the eyes and muscle pain On Aug 1 there was an acute tonsillit s



Fig 1 Central part of surgical specimen of lung with fibrosis inflammatory reaction and multinucleated giant cells Haematoxylin-eosin × 160

and the patient was treated with penicillin In spite of this a typical erysipelas appeared on Aug 25 on the no e and cheeks and the temperature was above 38 C On Aug 27 there was an erythematous multiform like rash in the left palm and lower arm The treatment was changed from penicillin to oxytetracycline and on Sept 2 the patient was afebrile and the rash was gone

Third admission The patient was admitted to the Department of Infectious Diseases on Oct. 5 1964 after having been subfebrile for 14 days with a temperature around 38 C headache and conjunctivitis A tender lymph node as also found in the right submandibular space The ESR s 88 mm and the Hb 65 On admission it was noted the patient seemed fatigued and very pale

X ray of the chest on Oct 13 1964 Small right sided stoperative pleural adhesions as in earlier examinations this year (Right lower lobe was resected on July 10 1963) In the basal posterior part of the right lung dense widespread infiltrates were observed In the perihilar region the changes had a more mottled appearance with small opacities of varying size No changes in the left lun The changes corresponded well with a pneumonia.

Three days later a new chest radiogram revealed a progress of the parenchymal changes also to the subapical parts of the right lung Still no changes in the left lung The heart was of normal size and shape The pulmonary vessels were normal Pneumonia was still the most probable radiological diagnosis

On Oct 16 penicillin treatment was instituted and the patient s condition then grew worse She was more pale there was a new process in the right lung and the patient had daily haemoptyses

The Hb was 7 6 g NPN 40 mg serum creatinine 1 6 mg Macroscopical haematuria with both h a line and granular casts was noted The ESR was 100 mm serum iron 35 μg and TIBC 232 μg

During her first stay in the Department of Infectious Diseases the patient was treated with penicillin and chloramphenicol No corticosteroid treatment was given She was transferred to the Department of Internal Medicin with the presumptive diagnosis of systemic lupus erythematosus

During the stay in the Medical Clinic the patient was



Fig Irregular fibrous thickening of alveolar walls Large amounts of red blood cells and haemosiderin-containing macrophages Haematoxylin-eosin × 128

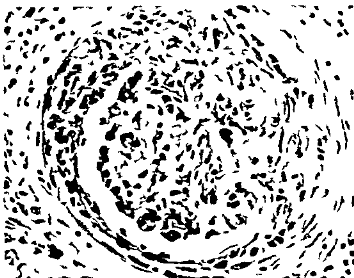


Fig 3 Glomerulus with epithelial prolifera tion and crescent formation Haematoxyl n eosin, × 360

treated with oxytetracycline Again steroid treatment was given. During the whole course the patient was subfebrile

The blood pressure was 130/80 the Hb 6 3 g the total eosinophile count 944 serum iron 54 μg % TIBC 247 μg% ESR 145 mm haptoglobin 350 mg% and serum creatinine 3 45 mg% Several red blood cells were seen in the urinary sediment. The ANF was ne ative No LE cells were found The AST was 360 I U

The patient grew still worse with nausea vomiting, increasing uraemia tendency to pulmonary oedema and daily haemoptyses She died on Oct. 10 1964

Pathological findings Besides a fatty li er of slight degree there were changes only in the lungs and the kidneys The lungs were solid especially on the right side (the right lung weighed 610 g the left lung 650 g) The cut surfaces showed dark reddish blue irregular areas with an increased amount of foamy fluid On both sides small apical fibrous scars were seen.

The kidneys had normal fat capsules (the right kidney weighed 110 g, the left kidney 130 g) The fibrous capsule was rather easily removed and the kidneys had a yellow brown surface with dilated vessels and petecchial haemorrhages The cut surface showed a cloudy picture with an unsharp border between cortex and marrow The colour was greasy yellow brown The pelvis and calyces were normal with pa e mucous membrane

Microscopically the lesions of the right and left lung were similar although more pronounced on the right side The picture was dominated by areas with large amounts of red blood cells in the alveoli Furthermore many

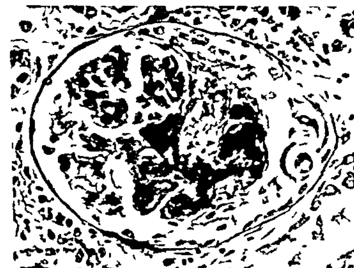


Fig 4 Glomerulus with fibrinoid necroses n some of the capillary loops Phosphotun s c ac d ha matoxylin × 400



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Fig 2 Irregular fibrous thickening of alveolar walls Large amounts of red blood cells and haemosiderin-containing macrophages. Haematoxylin-eosin × 178

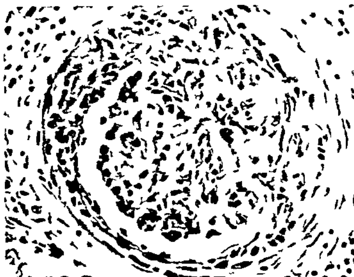


Fig 3 Glomerulus with epithelial prolifera tion and crescent formation Haematoxylin eosin, x 360

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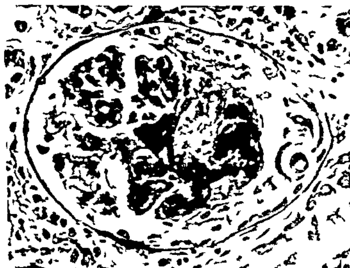


Fig 4 Glomerulus with fibrinoid necroses in some of the capillary loops Phosphotungstic acid haematoxylin 400

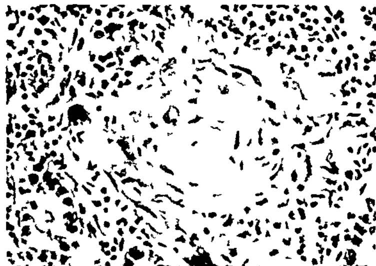


Fig 5 Destroyed glomerulus leaving fibrous scar with giant cells Haematoxylin-eosin, × 360

haemosiderin containing macrophages were seen both in the alveoli and in the alveolar walls many of which showed fibrous thickening The fibrosis was of the same type as that in the peripheral parts of the surgical speci men (Fig 2) Compact collagenous parts were not present No giant cells No fat positive substance No signs of vasculitis but in several mediumsized vessels thrombi were seen in some parts in organization Furthermore bronchopneumoniae were seen even in parts without haemorrhage The inflammatory cells were mostly inside the alveoli With PAS staining no di tinct basal membranes re seen in the alveolar walls only diffuse patches of ly stained material There was no splitting up of tic fibrils

In the kidneys there were more or less pronounced lesions in practically all glomeruli A few were apparently normal and a few showed prominent epithelial prolifera tion with crescent formation (Fig 3) In some there was fibrinoid necrosis involving only one or two capillary loops while in others more or less the whole tuft was necrotic The necrotic material was strongly stained in PAS and PTAH (phospho tungstic acid haematoxylin) (Fig 4) The necrotic tufts had been partly or totally replaced by fibrous tissue Some were hyalinized but the destruction in mo t glomeruli resulted in small and vaguely demarcated fibrous scars containing fibroblasts and a few giant cells (Fig. 5) In some of these scars small irregular rests of necrotic capillaries were seen (Fig 6) Tubuli were in some parts atrophic and some tubuli showed a hyaline droplet degeneration (Fig 7) Interstitially there were patches of inflammatory cells both plasma cells, lymphocytes and polymorphonuclear leukocytes There were no vascular changes except those described in the glomeruli

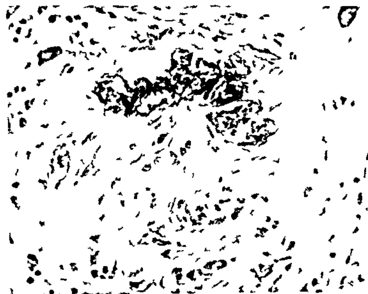


Fig 6 Scar with irregular rests of necrotic capillaries Giant cells in the periphery Periodic acid Schiff × 400



Fig 7 Tubulus with hyalin droplet degeneration. Periodic acid Schiff × 400

DISCUSSION

The lung lesions in Goodpasture s syndrome can not be separated clinically radiologically or pathologically with certainty from those seen in idio pathic pulmonary haemosiderosis (I P H) a disease which may be complicated by glomerulone phritis Heptinstall and Salmon (10) studied 69 cases of I P H and found five with histologically proved kidney lesions of focal glomerulonephritis type In one of these cases there were changes similar to those found in periarteritis nodosa (P A N) They also found three cases with clini cal signs of kidney disease but data of the kidneys were lacking in the autopsy records It is very un likely for many reasons that all cases with the combination of pulmonary haemorrhage and glom erulonephritis would be an accidental coincidence of I P H and glomerulonephritis The combination is more common than to be attributable to mere chance and I P H is predominantly a disease of children of both sexes while Goodpasture s syn drome is usually seen in younger men In most of the cases of Goodpasture s syndrome when kidney biopsies have been performed the glomerulone phritis in the earlier stages has been found to be focal and local but in a very short time it may become diffuse in patients who live long enough (1 9 11 17 18) Such focal nephritis is often seen in diseases which are considered to be of auto-immune type e g collagenoses (P A N systemic lupus erythematosus and Schonlein Henoch s pur pura) but these diseases do not have the same distribution in sex and age as Goodpasture s syn drome In the so-called recurrent haematuria or isolated haematuria a focal glomerulonephritis is found and this disease has the same age and sex distribution as Goodpasture s syndrome In these cases however the disease starts with a haematuria which is usually profound and the attacks return at intervals of weeks months or years Proteinuria is not a constant finding but may develop after several attacks This disease often starts in con nexion with or immediately after an infection usually in the upper respiratory tract and the interval which is typical of the classical post streptococcal glomerulonephritis is missing No definite relation to a streptococcal infection has been proved and a clinical picture of this type is not commonly found in Goodpasture s syndrome

It has been suggested that a streptococcal infec tion should be the cause of Goodpasture s syn drome (15 20) Other authors (17) do not think this likely They do not find anything specific at kidney biopsy and the picture is not similar to that usually seen in poststreptococcal glomerulone phritis

Benoit et al (1) have proposed autoimmunity as the cause of Goodpasture s syndrome and that this autoimmunity might be provoked by a viral infection They base this suggestion on the case described in 1919 by Goodpasture a young man who suddenly fell ill with pulmonary haemorrhage and glomerulonephritis after an attack of epidemic influenza The other fact put forward by Benoit et al (1) is that most of the cases of Goodpasture s syndrome described started in the years 1957 and

1958 when there was a simultaneous pandemic of influenza There is however no definite proof of autoimmunity started by a viral infection Cultures from three patients have yielded no viral growth and no viral antibodies have been found Duncan et al (5) demonstrated viruslike bodies by electron microscopy but Rohr et al (17) were not able to confirm this Lexow and Sigstad (14) found no antibodies to kidney tissue Experimentally however it has been shown that lung antibodies might give rise to nephritis through a cross reaction (4) and common antigens in both epithelial and mesenchymal basal membranes in different tissues have been demonstrated (12 13) According to some authors (1 17) cases which show inflammatory changes of the vessels should not be considered as Goodpasture s syndrome According to Heptinstall (8) however they should belong to this group He states that there is no sharp limit in the histological picture between cases with and without vascular changes and in the case of Goodpasture s syndrome from 1919 vascular lesions were found in the gut and necroses were seen in the spleen in connexion with the blood vessels

The histological picture has been described in the case reports In two of the cases the kidney lesions were very advanced and showed the pic[illegible] of a diffuse glomerulonephritis with prac[illegible]ally all glomeruli hyalinized Only a few glomer[illegible] were somewhat better preserved but in these as well there was hyalinization in most of the tuft Adherences were seen In some places there were epithelial proliferations with crescent formation In case 3 however focal glomerulonephritic changes were seen in different stages and of the same type as that described by other authors in early stages of Goodpasture s syndrome Benoit et al (1) considered the description fibrinoid necrosis of the lesions in the capillary tuft incorrect because they did not have the tinctorial properties of fibrin (PAS and PTAH staining) These stainings were however positive in our case 3 the only case which also had earlier changes at autopsy In this case too foreign body giant cells were seen around destroyed glomeruli This has earlier been described by Cruickshank and Parker (3) None of the three cases showed inflammatory vascular changes

The pulmonary lesions were of the type described in I P H Thus scattered intra alveolar haemorrhages were found and several haemosiderin containing macrophages in the s pta as well as in the alveoli In all cases there was a fibrous thickening of the alveolar septa most pronounced in case 3 who had had a localized process in the right lung one year earlier In all cases pronounced inflammatory changes of the type terminal bronchopneumonia were found No necrotizing alveolitis as described by Parkin et al (16) was found Elastine stained s ctions did not show any splitting up and there were no signs of vasculitis In PAS stained sections no distinct basal membranes were found in the alveolar septa However patches of vaguely stained PAS positive material were seen in the alveolar walls Rohr et al (17) demonstrated similarities in the lesions of kidneys and lungs The lesions were localized predominantly on the capillary side of the basal membrane They suggest that the lesion has a great similarity to antigen antibody reaction Case 3 is of special interest because of the unusual course which gives the impression of a case of Goodpasture s syndrome appearing in two distinct bouts but with some differences both clinical roentgenological and histopathological As a sign of kidney affection there was in the first bout only a microscopic haematuria although the kidney function was normal The lung lesion was characterized by a localized process in the right lower lobe Histopathologically there was a pronounced fibrosis with multinucleated giant cells of foreign body type Otherwise the changes were of principally the same type as at autopsy one year later Clinically there were no haemoptyses but both in sputum and in the surgical specimen haemosiderin containing macrophages were seen Furthermore macrophages were seen containing fat positive substance but the picture had no similarity to lipid pneumonia in other aspects A similar picture has been described by Botting et al (2) On electron microscopic examination of lung tissue they found material which was thought to be lipid or lipoprotein The cause of this localized haemosiderosis is not clear After lobectomy the haematuria disappeared And if one considers Goodpasture s syndrome to be an autoimmune process it is possible that by removal of changed lung tissue the production of antibody was stopped or depressed and at least for a while retarded the progress of the disease

As the aetiology and pathophysiology of Goodpasture s syndrome is not known in detail there is

no rational treatment In the literature there are reports of cases who have survived the disease but most have died Some authors claim that steroid treatment is the treatment of choice because the disease is considered to be an immunopathy A temporary improvement connected with steroid treatment was seen by us in both case 1 and case 2

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PERNICIOUS ANAEMIA PARAPROTEINAEMIA WITH UNUSUAL FEATURES AND CHROMOSOME ABERRATIONS

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Abstract A case of paraproteinaemia associated with pernicious anaemia is described. The patient s serum con tained 2 M-components (IgM and IgG) while the urine contained gamma globulin of the L-chain type All ab- normal components belonged to type Kappa Chromosome studies on blood cultures revealed a mosaic pattern about one third of the cells having a karyotype identical to that found in Down s syndrome (trisomy of a small acrocentric chromosome) the remainder having a normal male con stitution

While a megaloblastic erythropoiesis has occasion ally been described in myeloma patients (13) true pernicious anaemia appears to be rare in this con dition Mandema (24) reported one case and Larsson (20) four cases In two instances myeloma and pernicious anaemia were observed in the same family (18 23) No reports on the coexistence of pernicious anaemia and Waldenstrom s macro- globulinaemia have been found in the literature

In some cases of paraproteinaemia unusual fea tures have been described for example the presence of more than one M-component in serum (9 19) or chromosome aberrations (4)

The purpose of this paper is to report a case of pernicious anaemia exhibiting two different M components in serum excretion of light chains in the urine and chromosome aberrations

MATERIAL AND METHODS

1 Case History

The patient was a married gardener born in 1889 The patient could give no information concerning cases of blood dyscrasias or chromosome abnormalities, especially Down s syndrome in the family

When he was 73 years old he (V H) was admitted on account of general lass tude pain in the tongue acro- paraesthesias and a loss of weight of ten kg in six months On admission the patient appeared pale and emaciated. The tongue was smooth and red, but uvula and soft palate were normal No jaundice was observed patellar and Achilles-tendon reflexes as well as vibration tests were normal Lymph nodes liver and spleen were of normal size Bone tenderness bone protuberances, purpura or Raynaud phenomena d d not occur

Paraproteinaemia and pernicious anaemia were diagnosed. Antipernicious treatment was started with a vitamin B_{12} depot preparation (Betolvex®) 1 mg i m once a week for five weeks Thereafter the patient was given vitamin B i m every 4th week. After treatment for eight weeks the haemoglobin concentration was normal The patient s paraproteinaemia was not treated

The patient was controlled nine times during 3 / years His general condition remained good and haemo- globin levels have been normal No clinical signs of mul tiple myeloma or Waldenstrom s macroglobulinaemia have been detected although the ESR has consistently been elevated and the paraproteins in blood and urine have been unaltered.

Laboratory tests

Blood WR neg. Hb 6.5 g 100 ml, ESR 151 mm/h erythrocytes 1 57 mill /μl colour index 1 43 thrombo- cytes 240 000/μl, leukocytes 4 360 μl the differential count showed a slight lymphocytosis. The neutrophils were hyper segmented. The red blood picture showed megalocytosis and a marked anisocytosis and poikilocytos s No plasma cell were found in peripheral blood Serum B_{12} level was 60 pg/ml, serum folic acid 0 008 μg ml serum iron 146 μg 100 ml and serum TIBC 304 μg/100 ml

U ne No protein could be detected by conventional methods Tests for glucose blood and urobilin were nega ti e Amylase 3 units Xylose test 10 4 g was excreted during .4 h after ingestion of 75 g xylose Schilling test without intrinsic factor urinary excretion % of the radioactive B_{12} dose Schilling test with intrins c factor showed a . 0% e cretion

Faeces E ght samples gave negative benzidine reaction

Gast ic j ice Negative Congo reaction 60 min after histamine njection



Fig 1 (a) Abnormal plasma cell with flaming of the cytoplasm (× 1 400). (b) Abnormal plasma cell with thesaurocyte-like appearance eccentric somewhat pyknotic nucleus and large homogeneous cytoplasm divided into compartments by thin basophilic trabeculae (× 1 400)

Biopsy from mucous membrane of rectum No sign of amyloidosis (sign Prof G Teilum)

Retina Retinopathia paraproteinaemica

X-ray examinations No osteolytic lesions or diffuse halisteresis were found either in the cranium costae columna pelvis or the long bones Intravenous urography and examination of colon with contrast showed normal conditions Heart and lungs were normal but a slight atherosclerosis was noted in the aorta. Examination of jejunum and ileum with contrast showed a slightly flocculent appearance

Other routine laboratory studies Serum bilirubin 0 4 mg/100 ml prothrombin proconvertin time after Owren 85 % serum sodium 147 mEq/l serum chloride 100 mEq/l serum potassium 4 7 mEq/l serum creatinine 0 7 mg/100 ml standard bicarbonate 24 7 mEq/l serum glutamic-oxalacetic transaminase alkaline and acid phosphatase all showed normal values LE-cell phenomenon did not occur serum cholesterol 17 mg/100 ml ECG normal

Sternal marrow Cell content 360 000/μl Megaloblasts and many giant stabs were found Only 2 plasma cells were present several plasma cells showed flaming cytoplasm (Fig 1 a) Few thesaurocytes (Fig 1 b) were observed Differential count revealed 20 lymphocytes and 2 reticulum cells Many of the lymphocytes had very little cytoplasm but seemed otherwise normal (sign Prof G Gormsen)

Protein examinations Total serum protein 8 3 g/100 ml. Paper electrophoresis showed an M-component with gamma mobility contributing 19 5% of the total protein.

Immunoelectrophoresis (IE) IE was performed as described by Clausen (7) In all cases 1.5 μl antigen sample was used In all studies 80 μl antiserums were used (vide infra) Two paraproteins were present an IgG and an IgM type (Fig 2a) The IgM paraprotein precipitated weakly with anti IgA antiserum

2 Methods for Chromosome Investigations

Chromosome preparations were made from short term cultures from peripheral blood on two occasions. The

method was essentially that described by Moorehead et al (6) Skin cultures were also studied (14) An attempt to study the chromosomes in a direct preparation from bone marrow was unsuccessful

3 *Methods for Protein Investigations*

The paraproteins present in serum (diluted initially twice with distilled water) and urine (con entrated 100 times in vacuum dialysis) were prepurified by stepwise ammonium sulphate precipitation (pH 6 8) and isolated by Sephadex G 00 filtration After concentration to 2 g/100 ml by vacuum dialysis the fractions obtained were also studied by a ar gel micro-electrophoresis. Light chains (Kappa or Lambda) were identified in IE of concentrated protein peaks obtained by filtration on Sephadex G 200 of whole serum and urine concentrated 100 times by vacuum dialy sis

Vertical starch gel electrophoresi was performed in 8 M urea and formic acid buffer at pH 3 0 The purified M-components from serum and urine were reduced by means of β-mercapto-ethanol and alkylated by means of iodoacetamide (16)

Immunologically pure human IgG globulin was used as reference standard for H and L chains after reduction and alkylation The IgG globulin was isolated from 2 00 ml human serum by column chromatography on DEAE cel lulose (dimensions 1×30 cm) The first eluted peak, con centrated to 5 g/100 ml was shown by IE to contain only the slow migrating IgG globulin

The reagent grade chemicals used were all of highest obtainable purity from British Drug House

Examination of the hand and foot prints was performed by L S Penrose London and the identification of Kappa chains by H Olesen Bispebjerg Hospital Copenhagen

RESULTS

1 Cytogenetical observations The patient was chromatin negative as determined on Feulgen stained buccal smears The results of blood cul tures (on three separate samples within four months) and on skin cultures at the same time as the second blood culture are shown in Table I

All cells with 46 chromosomes contained five

Table I *Chromosome count distribution on blood and skin cultures from case V H*

	Chromosome count distribution					
	<45	45	46	47	>47	Total
Blood I	1	1	16	7	—	25
Blood II	1	—	21	13	—	35
Blood III	—	5	12	3	—	20
Total blood	2	6	49	23	—	80
Skin	—	2	28	—	—	30

small acrocentrics In nearly all cells the Y was discernible In one of the 47 chromosome cells the extra chromosome was medium sized subme a centric

Analysis of cells with 46 chromosomes revealed a normal male karyotype Cells with 47 chromo-somes showed a normal male karyotype and an additional small acrocentric chromosome indistin guishable from the other members of the group 21–22

2 Dermatoglyphic examination There was no marked indication of mongolism in the lines of the hands or fet The sum of the maximal at angles was 93° the mean for control males is 85° ± 15° and for mongol males 137 ± 27°

3 Results of protein investigations Fig 2*a* demonstrates the finding of 1 5 μl serum applied in IE. By means of an antiserum (80 μl) from horse against pooled normal human serum the precipitation arc for the IgG globulin in the β 2 area deflects towards the antibody trough This deflection can be seen more distinctly in immuno-electrophoresis with a rabbit antiserum (80 μl) strictly specific to the IgG globulin Furthermore the abnormal arc coalesces with the normal γ globulin arc The deflection of the IgG globulin in immuno-precipitate indicates an excess in a well

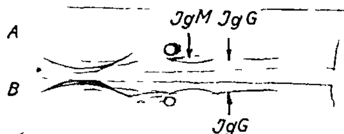


Fig 2a Immuno-electrophoresis of the pathol gical serum (upper filling hole *A*) correlated to that of normal serum (lower f l n hole *B*) The electrophoresis is devel oped w th n antiserum against whole hu man serum (horse antiserum) Two anoma lous arcs are seen with patholog cal pattern one corresponding to the IgG globul n and one to the IgM globulin.

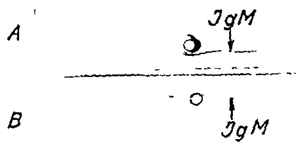


Fig 2b Immuno-electrophoresis of the pathological serum (upper filling hole *A*) correlated to that of normal serum (lower filling hole *B*) The electrophoresis is developed with an antiserum against IgM macroglobulin A double humped arc corresponding to the anomalous IgM globulin is seen. A weak striation phenomenon cathodic to the filling hole is also demonstrated

defined mobility interval of the corresponding protein antigen of paraprotein character No Bence Jones protein was noted

Furthermore in and cathodically to the central filling hole a dense coloured zone can be seen This is an indication of a high molecular component (molecular weight above 200 000) which cannot penetrate through the micelle structure of the gel Furthermore a weak but distinct precipitate around this zone can be seen However by treatment of the serum with 0 5 % neutralized cysteine a rabbit antiserum specific to IgM will now give rise to the appearance of a distinct precipitate for this immunoglobulin (Fig 2*b*) This precipitate deflects towards the antibody trough as a sign of a localized excess of an IgM paraprotein in the mobility interval cathodically to the central filling hole

Agar gel micro-electrophoresis reveals two distinct M component bands most easily seen in electrophoresis of the dialysed and concentrated (2g/100 ml) supernatant from 1 25 M ammonium sulphate precipitation (Fig 3) Cathodically to the transverse filling trough a distinct dense zone of protein can be seen corresponding to the IgM paraprotein More cathodically another abnormal M-component appears (IgG paraprotein)



Fig 3 Agar gel microelectrophoresis of pathological serum precipitated in the range from 1.2–1 9 M ammonium sulphate Two abnormal fractions are seen corresponding to the IgM and IgG globulin.

Immunoelectrophoresis of urine (1 5 μl) concentrated 100 times by vacuum dialysis revealed precipitates for albumin transferrin and an arc situated in the β 2 area close to the antibody trough This arc develops in IE with an antiserum specific to IgG

Ammonium sulphate fractionation and agar gel microelectrophoresis of whole serum revealed 80 % of the paraprotein components to be precipitated in the interval from 1 25 to 2 20 M ammonium sulphate (pH 6 8)

The abnormal IgG globulin free of IgM was precipitated in the interval of 1 25 to 1 90 M ammonium sulphate The precipitate obtained in the range of 1 9 to 2 2 M free of IgG was used as a source for isolation of immunologically pure IgM component Sephadex filtration of this fraction (Sephadex G 200) gave purified IgM component (98 % pure state) as the first small sharp peak

The IgG from the urine was isolated by column chromatography on DEAE cellulose of twenty four hours concentrated and dialysed urine Batches of 1 ml dialysed and concentrated urine (4 5 g protein/100 ml) were chromatographed The IgG fraction was isolated by elution with low ionic strength (2000 to 5000 μ Siemens conductivity)

Immunologically pure human IgG the isolated IgM paraprotein and the isolated urinary IgG fraction were reduced and alkylated After dialysis of the fractions against 8 M urea the reduced and alkylated fractions were subjected to vertical starch gel electrophoresis Fig 4 demonstrates the

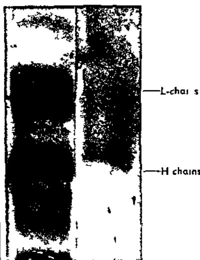


Fig 4 Starch gel electrophoresis of anomalous IgM globulin isolated from serum (to the left) correlated to abnormal component isolated from urine (to the right) The fractions isolated were reduced and alkylated prior to the electrophoresis (see text)

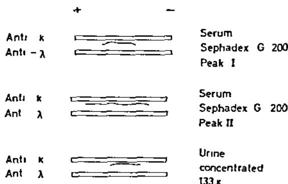


Fig 5 Immunoelectrophoresis with antisera against Kappa and Lambda chains after fractionation on Sephadex G 200 Peak I contained IgM globulin Peak II the IgG globulin and the urine IgG globulin (see text)

results The IgM paraprotein reduced and alkylated gave two main bands in the starch gel corresponding to the L and the H chains Some less well stained bands were apparent The reduced and alkylated urinary IgG revealed only two protein bands one with mobility overlapping the faster part of the H band and one corresponding to the L-chains No other bands could be traced

Immunoelectrophoresis of concentrated urine and peaks from Sephadex G 200 filtration of serum revealed only the presence of Kappa L chains (Fig 5)

DISCUSSION

Cytogenetical discussion

In cases of paraproteinaemia abnormal cell lines with an extra large meta or submetacentric chromosome have been described in peripheral blood (3 11 12 15 32)

Castoldi et al (5) found a modal number of 44 chromosomes only in bone marrow cells in a case of multiple myeloma The karyotype seemed variable In three cases of the same disease Lewis et al (21) found a mixture of normal and hyperdiploid cells

Bottura (4) examined five cases of multiple myeloma for chromosome abnormalities One of these patients suffered from an α myeloma and chromosome studies on bone marrow cells revealed two lines one with a normal male karyotype and one with 45 chromosomes The abnormal cells all lacked a small acrocentric chromosome while the Y chromosome was present

The karyotype found in a proportion of the patient s blood cells was identical with that described in Down s syndrome (mongolism) The question arises whether this case is one of paraproteinaemia in a patient with a chromosome mosaicism of normal cells and cells with a 21 trisomy An answer can probably never be given

The reports on chromo ome mosaicism in Down s syndrome are few Clarke et al (16) found a mosaic in skin cells but normal cells in blood In some other cases blood cells alone have been studied and chimera like conditions as found in our patient may thus have been overlooked All authors found some mongoloid clinical features in their patients Blank et al (2) found a mosaic in the clinically normal mother of a patient with Down s syndrome

The present findings did not indicate Down s syndrome and this disease was absent among the relatives Therefore there may be a connection between the chromosome abnormality and the observed protein abnormality

The abnormal cells lines cannot be caused by cytotoxic agents or blood transfusion since such treatments were never given

In the case of an α myeloma described by Bottura (4) a member of the 21–22 group was also involved though lacking in contrast to our case

Clinical protein chemical and histological discussion

Low levels of vitamin B_1 in serum and/or deficiency of folic acid are known in patients with myeloma (20 24 25) primary macroglobulinaemia (8) and other neoplastic diseases (30) The vitamin B_1 deficiency in these cases has been explained by consumption of vitamin B_{12} by the growing malignant cell populations Furthermore transport of vitamin B_1 due to abnormal or lacking plasma proteins may give rise to megaloblastic anaemia (17) but these patients do not respond to vitamin B_1 therapy

In the present case serum bilirubin and serum iron showed normal values but otherwise the clinical findings and laboratory data suggested a typical pernicious anaemia

The chemical investigations indicate the presence in serum of two M components as previously described by Drivsholm and Clausen (9) and Kjeldsen and Asfeldt (19) The case story is difficult to evaluate as primary macroglobulinaemia and multiple myeloma exhibit a number of similar sign A precise diagnostic classification of some cases of M components is difficult or impossible (28 33)

The use of electrophoresis as a routine laboratory procedure unveils cases with M components which initially do not represent myeloma or primary macroglobulinaemia Some of these cases later develop clinically typical myeloma or macroglobulinaemia (27) There remain however a number of cases with M-components which fail to develop an identifiable clinical pattern (33 34) Usually these so-called monoclonal gammapathias are an IgG type protein abnormality although IgA and IgM exist

In primary macroglobulinaemia the Bence Jones protein excreted in the urine belongs to the L-chain type which also is found in most patients with multiple myeloma (10 16) In the present case the gamma globulin component present in the urine seems similarly to be of the Kappa-chain type which also was found in the serum paraproteins

A correlation between the immuno-electrophoretic findings in serum and urine and the morphological characteristics of the cells responsible for their production has been reported (9 29 33) The flame cells and "thesaurocytes are thus exclusively found in IgA myelomas The formation of IgM protein usually takes place in the lymphoid reticulum cells (1)

The present case seems to be another one of the still more frequent obscure clinical pictures combined with the presence of M-components in serum and/or urine Instead of attempting a precise clinical classification it may be more satisfying to classify these patients according to their protein chemical abnormality

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Acta med scand Vol 186 pp 217–222 1969

LACTASE DEFICIENCY AND A LACTOSE FREE DIET IN PATIENTS WITH UNSPECIFIC ABDOMINAL COMPLAINTS

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Abstract Jejunal lactase activities have been determined in 41 patients who had consulted general practitioners on account of unspecific abdominal complaints Twelve of these patients had low jejunal lactase activity and they all had selective lactase deficiency

A lactose free diet for two weeks had a beneficial effect on all the patients with low lactase activity and the effect was not temporary as could be noted on inquiry about six months later

It is remarkable that only two of the twelve patients w th low lactase activity had a history of milk intolerance

Determinat on of small intestinal lactase activity or a peroral lactose tolerance test is recommended as a routine test for milk-drinking patients with unspecific abdominal complaints

According to a recent interview survey abdominal symptoms and diseases are the third most common group of chronic diseases in the adult population in Finland and about 1 5 of those interviewed suffered from abdominal symptoms without any demonstrated organic disease (14) This problem may be worldwide for in the U S A digestive diseases ranked fourth as a cause of total economic cost of illness grouped by major diagnostic cate gories (2) and they were the second most common cause of disability among a selected group of employees (4) On the other hand it is well known that small intestinal lactase deficiency is rather common and causes diarrhoea abdominal fullness and meteorism after ingestion of lactose-containing foods almost the same symptoms as are reported by patients with unspecific abdominal complaints" and with nonspecific gastroenteritis and diarrhoea In accordance with this it has been shown that many hospital patients with functional diarrhoea (12) and irritable colon syndrome (15) had lactase deficiency and their symptoms diminished on a lactose free diet

Lactose intolerance is an important problem in countries where milk consumption is high Milk consumption in Finland during 1965 was 290 kg per head (11) one of the highest in the whole world and about twice as high as in the U S A (6)

It therefore seemed especially necessary to clarify

1) the frequency of lactase deficiency in patients with unspecific abdominal complaints visiting general practitioners

2) the effect of a lactose free diet on the symp toms of these patients

This study deals with these problems

SELECTION OF PATIENTS

From the consulting rooms of the two doctors taking part in the investigation were remitted those patients with abdominal complaints who in X ray and laboratory examinations had been verified not to have any organic disease of the digestive organs and who in addition to this also fulfilled the fol lowing criteria

1) patients had at least one of the following symptoms abdominal fullness feeling of nausea unidentified abdominal distress meteorism loose stools watery diarrhoea

2) patients had the symptoms on average at least once a week and had had them at least more than one month before consulting the doctor

3) patients sought medical aid because of ab dominal distress or spontaneously mentioned the symptoms during the examination or treatment of some other disease

4) no other medicament influencing abdominal symptoms than Enzynorm® tablets were necessary during the experiment

MATERIAL

Altogether 43 patients who fulfilled the above criteria visited the two general practitioners taking part in the investigation during the period January to May 1968 Two refused small intestinal biopsy and were not included in the study Small intestinal specimens were taken from 41 patients 32 of whom were women 27 to 62 years of age (mean 47 5) and 9 men 28 to 6? (mean 41 5 years)

METHODS

Design of diet experiment

During the study the patients were

one week on a lactose free diet (week I)
one week on their customary diet (week II)
one week on a lactose free diet (week III)

During the lactose free diet foods containing milk and lactose were forbidden However cheese butter and sour milk products were allowed because in cheese and butter lactose is only presented in minimal amounts if at all and in sour milk products lactose is already partly hydrolysed (9) and probably lactose splitting bacteria continue their function in the stomach and small intestine where an optimal temperature for their function prevails

The customary diet did not differ from the food habits of the patients

The effect of diet was estimated by patients weekly comparing subjective abdominal symptoms and consistency and frequency of stools to the corresponding ones of the previous week In this way the effect of a *short term lactose free diet* was noted The patient and the treating doctor were not informed of the results of lactase determination until the diet experiment was over

Small intestinal biopsies

Small intestinal biopsies were taken from the jejunum immediately distally of the ligament of Treitz with Bult s or Brandborg Rubin s biopsy instrument The specimens were cut in half and one half was fixed immediately in 10% neutral formalin stained in haematoxylin eosin and examined under the light microscope The specimens were classified as normal if four normal villi were found adjacent to each other The amount of round cell infiltration *in the lamina propria of the small intestinal mucosa* was roughly estimated The other half was stored at −20 C until determination of disaccharidase activities according to a modification (10) of the original method of Dahlqvist (5) The disaccharidase activity is expressed as units/g mucosa wet weight The lowest limit of normal lactase activity with the method used is 1.5 units and that of the normal lactase to sucrase ratio is 0 3 The limits are estimated on the basis of 89 consecutive structurally normal biopsies the analysis of which according to McMichael (12) is presented elsewhere (7)

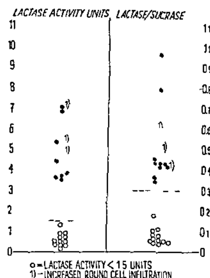


Fig 1 Distribution of jejunal lactase activity and lactase to sucrase ratio in the patients investigated

Later inquiry

In October 1968 six months or so after the diet experiment a follow up inquiry was made The patients were asked whether they had abdominal complaints and whether they had continued with the lactose free diet In this way it was possible to notice the effect of the *long term lactose free diet* on some of the patients investigated In the case of symptomless patients still on the lactose free diet, the patient was asked to drink a glass of milk twice a day during meals for three consecutive days The harmful effects of milk were then to be reported In the later inquiry information was received from 39 patients

RESULTS

Small intestinal biopsies

None of the patients had villous changes but three had increased round cell infiltration in the lamina propria of the small intestine Of altogether 41 patients examined 12 had low jejunal lactase activity (less than 1 5 units) and all of these but none of the others had a low lactase to sucrase ratio (less than 0 3) as shown in Fig 1

Subjective symptoms

Patients with low lactase activity did not differ from the others in regard to the nature and duration of the abdominal symptoms as shown in Table I

Table I *Abdominal symptoms and their duration in the patients investigated*

	Normal lactase activity				Low lactase activity			
	Duration of symptoms		No of pats	Symptoms of pats	Duration of symptoms		No of pats	Symptoms of pats
	<12 mo	>12 mo			<12 mo	>12 mo		
Abdominal fullness	5	24	29	100	2	9	11	92
Meteorism	4	18	22	76	2	8	10	83
Nausea	5	11	16	55	1	5	6	50
Unidentified abdominal complaint	3	10	13	45	1	4	5	42
Loose stools	2	9	11	38	2	3	5	4
Watery diarrhoea	3	1	4	14	1	2	3	25
Obstipation	1	4	5	17	–	1	1	8

Three of those investigated had a history of milk intolerance and two of them had low lactase activity

Short term lactose free diet

The short term lactose free diet had a beneficial effect on all the patients with low lactase activity and on some of the patients with normal lactase activity as shown in Table II The mean lactase activity of the patients with normal lactase activity noting a beneficial effect of the short term lactose free diet was 4 1 and that of the others with normal lactase activity was 5 0 (Fig 2) The difference is not statistically significant

The mean age of the patients with a low lactase activity was nearly the same as that of patients with normal lactase activity 47 5 and 46 years respectively

Later inquiry

According to Table III all the patients with low lactase activity were without symptoms at the later inquiry and they had all continued a lactose free diet for more than six months after the beginning of the experiment After drinking two glasses of milk daily for three consecutive days eight of them reported abdominal complaints while three remained symptom free Of the patients with normal lactase activity only eight were symptom free at the time of later inquiry Of these symptom free patients two had been subjected to gall bladder operation during the time after the short term lactose free diet and before the later inquiry and three others attributed their freedom from symptoms to the regular use of Enzynorm® tablets

Five of the 28 patients with normal lactase activity still continued on a lactose free diet only one

Table II *The effect of the lactose free diet on the abdominal symptoms in the patients investigated*

	Abdominal symptoms aggravated	Abdominal symptoms unchanged	Abdominal symptoms lessened	Abdominal symptoms totally disappeared	No of pats
1 two-week lactose-free diet					
Normal lactase activity	1	19		[illegible]	29
Low lactase activity	–	2	8	2	1
2 day-week customary diet					
Normal lactase activity	14	9	3	1	27[a]
Low lactase activity	10	–	–	2[b]	1[illegible]
3 day-week lactose-free diet					
Normal lactase activity	3	10	7	2	21[a]
Low lactase activity	–	–	6	4 – 2[b]	12

[a] Some of patients abandoned diet experiment
[b] Patients refused to drink milk

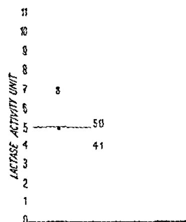


Fig 2 Distribution of jejunal lactase activity in patients *with normal lactase activity and the effect of a short term* lactose free diet Symbols ○ = short term lactose free diet had a beneficial effect --- the mean of these patients ● = short term lactose free diet did not have a beneficial effect ——— the mean of these patients

of these five had no symptoms and she had abdominal complaints when she started to drink milk again

The milk habits of the patients were not recorded but six patients with low lactase activity ~~ted that they had drunk unusually large amounts ~~ milk during the period before the experiment ~~ieving that milk would be beneficial for their abdominal symptoms

CASE REPORTS

Two case reports are given which illustrate the good effect of the lactose free diet

Case 1

A 34 year-old engineer who for more than four years had had upper abdominal pain nausea and diarrhoea He had a history of milk intolerance In 1966 X ray examination of the stomach revealed deformatio bulbi Anticholinergic medication did not help In 1967 the patient visited the doctor three times because of abdominal complaints In 1968 X ray examination revealed mild gastritis, the gall bladder was roentgenologically normal as was the result of aspiration of duodenal contents after secretin stimulation The patient s abdominal complaints disappeared during the short period on a lactose free diet and on later inquiry the patient was without symptoms and had continued with the lactose free diet The patient developed marked abdominal symptoms on starting to drink milk again Jejunal lactase activity was low

Case 2

A 41 year-old foreman s wife who has consulted many doctors several times during 5–6 years because of abdominal pain nausea and diarrhoea During these years the stomach had been examined roentgenologically three times and the gall bladder once every time with normal result The patient had been told to drink milk to relieve the abdominal complaints and she has been treated as a neurotic with tranquilizers The patient s abdominal complaints disappeared during the short term lactose free diet and at the time of the later inquiry she was without symptoms and had continued with the lactose free diet. The patient had no recurrence of abdominal complaints on drinking two glasses of milk daily during three consecutive days as requested Jejunal lactase activity was low

DISCUSSION

According to this investigation the frequency of lactase deficiency was 29 % in patients suffering from unspecific abdominal complaints and representing the general practitioners patient ma

Table III *The results of the later inquiry to 39 of the patients investigated*

	Abdominal complaints		Lactose free diet		Effect of milk		
	Present	No	Continued	Abandoned	Causes abdominal symptoms	Does not cause abdominal symptoms	No of pats
Low lactase activity	—	11	11	—	8	3	11
Normal lactase activity							
Short term lactose free diet beneficial	5	4[a]	5	4	1	–	9
Short term lactose free diet not beneficial	15	4[b]	—	19	–	–	19

[a] Two patients had gall bladder operation during time between short term lactose free diet and later inquiry
[b] Three patients attributed freedom from symptoms to regular use of Enzynorm® tablets

terial This generality corresponds to the lactase deficiency verified in hospital patients suffering from functional diarrhoea (13) and irritable colon syndrome (15) The frequency of lactase deficiency has been verified to vary considerably as a result of racial and genetic factors (1 3 12) which has to be taken into account in comparisons of investigations carried out in different countries

The significance of the frequency of this verified lactase deficiency still remains somewhat obscure because of lack of information on the general prevalence of lactase deficiency Some kind of standard of comparison is supplied by investigations carried out in Finland on selected hospital cases and on servicemen (8) According to this investigation 17 % of the hospital patients averaging 43 years and 18 of the servicemen averaging 20 years were verified to have lactose intolerance i e lactase deficiency Among the hospital cases 48 % of the patients with a history of milk intolerance 21 % of those suffering from repeatedly occurring abdominal complaints but without a history of milk intolerance and 7 % of those with no abdominal complaints or history of milk intolerance had lactose intolerance In 15 % of the servicemen who did not have abdominal complaints or a history of milk intolerance lactose intolerance was correspondingly verified

These facts suggest that there is no further increase in the occurrence of lactase deficiency once adult age is reached but that the deficiency becomes clinically manifest i e lactose intolerance appears at different ages especially in adult life This hypothesis i supported by the clear separation of the lactase population and lactase to-sucrase ratios into two groups without any intermediate forms as shown in Fig 1 and noted earlier (7 12) This being the case it is further very possible that when during the manifestation of lactose intolerance a patient with lactase deficiency continues to consume milk without noticing its harmful effects he becomes one of the patients with unspecific abdominal complaints This would also explain the fact that in our investigation only two out of 12 patients with unspecific abdominal complaints and lactase deficiency had a history of milk intolerance

The present investigation shows that it is important to diagnose lactase deficiency in patients with "unspecific abdominal complaints for this deficiency is common in them and the effect of a lactose free diet is indisputable The effect is not transient as was proved by the results of the later inquiry This also showed that after the symptoms had subsided some of the patients could take a small daily portion of lactose without developing symptoms The diagnosis of lactase deficiency in patients with unspecific abdominal complaints cannot be made on the basis of the case history or subjective symptoms because a history of milk intolerance is seldom associated with lactase deficiency by these patients and the symptoms are the same in patients with normal lactase activity Nor on the basis of the effect of a short term lactose free diet does it seem possible to diagnose lactase deficiency with certainty However failure of the abdominal complaints of the patient to decrease during a lactose free diet strongly suggests a normal lactase activity The diagnosis can be confirmed by determination of jejunal lactase activity or by a lactose tolerance test Because a satisfactory correlation exists between jejunal lactase activity and the result of a peroral lactose tolerance test the latter can be used as a routine test for milk-drinking patients with unspecific abdominal complaints

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HYPERPYREXIA IN ASSOCIATION WITH ADMINISTRATION OF L ALPHA METHYLDOPA

A Report of Two Cases

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Abstract Two cases of hyperpyrexia associated with administration of *l* alpha methyldopa are presented Febrile reaction has been reported to both D and L isomers of methyldopa but true hyperpyrexia has occurred only with the L isomer Previous attempts to obtain proof of an allergic reaction as a cause of hyperpyrexia have failed. In vitro studies were made of the leukocytes of one of the patients sensitized by administration of *l* alpha methyldopa, which was also used as an antigenic agent in the experiment The results suggest the possibility of toxic leukocytolysis a the causative mechanism underlying the hyperpyrexia.

Alpha methyldopa (alpha methyl 3 4-dihydroxy l phenylalanine) a decarboxylase inhibitor has a well-established position in antihypertensive therapy to-day The drug is thought to decrease the tissue stores of cathecholamines Although side effects are frequent they are usually mild and do not as a rule necessitate withdrawal of the drug The adverse reactions to methyldopa as reported in medical literature have already been extensively reviewed elsewhere (9) The major side effects mentioned in order of frequency and severity are liver toxicity fluid retention angina pectoris and febrile reactions Febrile reactions have usually been reported in a frequency of 1 to 3 % although as many as 6% have been encountered (5 6 7 11) Febrile reactions have been reported to both D and L isomers although true hyperpyrexia has only been described in connection with the administration of the L isomer (8) The febrile reactions have usually been mild and the temperature has not exceeded 40 C To our knowledge only one case of hyperpyrexia has so far been published (8) In the two cases to be described below there were hyperpyretic reactions in connection with the administration of the L isomer of methyldopa This type of reaction has been regarded as allergic (8) In order to test this hypothesis in vitro studies of the leukocytes of case 2 were carried out

CASE REPORTS

Case 1

The patient was a 4_ year-old male agriculturalist A transiently elevated blood pressure had been recorded in 1961 in connection with an operation on an intervertebral disc prolapse of the lumbar spine In June 1966 the patient was admitted to hospital because of intense headache impaired vision and elevated blood pressure (240 160 mm Hg) Examinations revealed bilateral astigmatism and grade IV hypertensive retinal changes The serum creatinine was slightly elevated 1 5 mg 100 ml Chest X ray showed dilatation of the left ventricle and corresponding ECG changes were recorded (left axis deviation and left side strain) Renal biopsy showed mild arteriolar hypertensive changes and some mild capsular adhesions in the glomeruli The patient received 750 mg *l* alpha methyldopa divided into three doses from July 7 1966 and in addition 40 mg furosemide daily The patient was discharged on Aug 8 1966 with this medication the blood pressure having dropped to 230 120 mm Hg with marked subjective improvement On Aug 5 1966 he had a temperature of 39 1 C haematuria and pyuria without bacteriuria and blood pressure 190 135 mm Hg The medication was stopped On Aug 10 1966 methyldopa and furosemide were reinstituted in the same dose and the temperature rose within two days to 40 C The patient was readmitted on Aug 13 1966 No signs of infection could be found Haemoglobin, however had fallen from 12 0 (Aug 10 1966) to 9 6 g 100 ml (Aug 19 1966) without any sign of bleeding or haemolysis normal bilirubin reticulocytes % elevated haptoglobin level direct Coombs test negative Furthermore there was a leukocytosis ad 11400 mm and eosinophilia ad 3 5% Methyldopa was once more withdrawn and the temperature returned to normal within a day In order to test the causative relationship between methyldopa and the febrile reactions the drug was r

instituted one and a half weeks later in a dose of 500 mg a day and the febrile reaction reappeared within three days It thus seemed highly probable that the hyper pyrexia was a side effect of methyldopa The patient was thereafter treated with guanethidine sulphate to which *furosemide and later hydralazine were added His blood* pressure respond d very poorly the renal function gradu ally deteriorating On Aug 9 1967 he was readmitted to hospital because of his greatly impaired general condi tion and a rise in the serum creatinine level from 4 4 to 11 0 mg/100 ml within a week The patient died on Aug 15 1967 from acute pulmonary oedema as a complication of uraemia and congestive heart failure Autopsy showed renal changes typical of malignant hypertension with *arterial necrosis No primary myocardial or pulmonary* disease could be detected

Case 2

A 52 year old woman was admitted to hospital in 1957 because of benign essential hypertension of 10 years standing as well as enlargement of the heart The initial systolic blood pressure had been 330 mm Hg The patient however became symptom free within some months on *oral antihypertensive therapy after which she abstained* from further medical supervision About ten years later a routine public health chest X ray revealed cardiac en largement on Febr 27 1967 Two months later she had a nocturnal attack of dyspnoea Her blood pressure was 270/140 mm at that time and she received rescinamine 125 mg chlorthiazide 250 mg and digoxine 0 25 mg once daily On July 6 1967 she consulted a physician The ollowing findings were made blood pressure 300/140 mm a regular pulse rate of 100/min an accentuated second sound and a grade II systolic murmur over the aortic valve no enlargement of the liver or peripheral pitting oedema grade I hypertensive retinal changes varicose veins of both legs weight 85 kg Chest X ray on July 13 1967 no parenchymal lung process or signs of cardiac congestion elongation of the aorta all round cardiac enlargement with total heart volume of 1200 ml equalling 640 ml/body surface sq m Laboratory findings serum creatinine 0 76 mg/100 ml Na 141 mEq/l K 4 3 mEq/l ESR 27 mm/1 h haemoglobin 13 3 g/100 ml leukocytes 7?00/mm urinalysis showed no protein or glucose pH 5 0 sp weight 1014 and in the sediment red cells 4/Hpf white cells 60/Hpf and Gram negative rods Therapy prescribed on July 6 1967 digoxine 0 25 mg chlorthiazide 500 mg *l* alpha methyldopa 750 mg divided into four doses On July 19 1967 the patient had diar rhoea and temperature ad 40 C After this she had several hyperpyretic reactions usually up to 40 C recurring ir regularly in the evenings The gastrointestinal symptoms rapidly subsided with diphenoxylate chloride and atropine sulphate Because of the febrile reactions the patient was r *ferred to* the hospital out patient department on July 25 1967 where the findings were blood pressure *230/130* mm Hg weight 84.5 kg no oedema She was admitted to the hospital ward on July 26 1967 The methyldopa treat ment was reinstituted (750 mg/daily) and there was an immediate rise in temperature ad 40 1 C The drug was withdrawn on the *following day and the temperature fell* to 38 4 C having returned to normal by July 31 1967

The same medication was repeated on Aug 8 and Au 10 1967 the patient reacting with temperatures of 39 4 C and 38 8 C respectively The hypotensive effect was satis factory the blood pressure falling to 145/100 mm Hg without other side effects apart from hyperpyrexia ac companied by leukocytosis (10 400/mm) with eosinophilia of 1000 cells/mm (July 28 1967)

On July 27 1967 ESR was 62 mm/1 h It was con cluded that the hyperpyretic reactions were associated with the administration of methyldopa With a regimen of Serpasil® 0 1 mg three times daily and 500 mg of chlor thiazide with potassium substitution the patient s hyper tension was sufficiently controlled (180/100 mm Hg) Digoxine was continued as before

LEUKOCYTE TESTS METHOD AND RESULTS

An attempt to clarify the mechanism causing the hyperpyretic reaction was made by studying the patient s (case 2) leukocytes in vitro Strong anti *gens are known to cause rapid destruction of* leukocytes in vitro in the simultaneous presence of both antigen and serum from sensitized indi viduals (1 3) Our intention was to record the in vitro reaction of the leukocytes from the pa tient sensitized by administration of *l* alpha methyl dopa which was also used as an antigenic agent in the experiment The white cell reaction was ob served before during and after the clinical hyper pyretic reaction Leukocytolysis is regarded as a phenomenon preceding febrile reactions (4 10)

The leukocytes were separated from the blood cells ac cording to the method of Boyle and Tuhkainen (2) L alpha methyldopa (Dopamet® A/S Dumex, *Denmark*) 250 mg was dissolved in 10 ml saline and serial dilutions were prepared from the stock solution One drop of the solution obtained was put in a specially *constructed* well slide and the solution allowed to dry The *suspension of* the patient s leukocytes and serum was pipetted to the dry antigen *The reaction of the leukocytes was recorded* half an hour later under the microscope before and after staining with methylene blue

The results of the leukocyte tests are presented in Table I It shows that the strongest solutions of methyldopa had a similar leukotoxic effect upon the white cells of the patient as upon those of the control Practically all cells were destroyed If the concentration of methyldopa was diluted *the leu*kocytolytic reaction became weaker and agglutina tion occurred This reaction too disappeared with the weakest solutions There was only a quanti tative difference between the leukocyte reactions of the patient and the control the leukocytes of

Table I *Leukocyte tests in vitro with l alpha methyl dopa*

Dilution	Cell agglutination	Devitalized cells ( )
The pat ent s white cell reaction before hype pyrexia		
1	None	100
1 2	None	100
1 4	None	100
1 8	None	90
1 16	None	50
1 32	Occurred	50
1 64	Occurred	50
The pat nt s white cell react on during hyperpyrexia		
1	None	100
1 2	None	90
1 4	Occurred	50
1 8	Occurred	20
1 16	Occurred	5
1 32	Occurred	5
1 64	None	5
Th pati nt s white ell reaction four days after normal ation of tempe atu e		
1	None	100
1 2	None	100
1 4	None	95
1 8	None	50
1 16	Occurred	50
1 32	Occurred	30
1 64	Occurred	30
Cont ol s bject s wh te cell rea tion		
1	None	100
1 2	None	90
1 4	Occurred	50
1 8	Occurred	15
1 16	Occurred	5
1 32	Occurred	5
1 64	None	5

the sensitized patient having only a lower tolerance the antigenic agent During the hyperpyretic reaction the patient s leukocytes however behaved like those of the control This is interpreted as evidence that the patient s sensitized white cells had undergone lysis in vivo immediately prior to the febrile peaks and that the sample taken during the febrile phase mainly contained still unsensitized leukocytes Four days later the normalized tolerance" was lost i e the reaction that of the sensitized pre febrile state

COMMENTS

From the data presented above the suggestion can be made that hyperpyretic reactions are associated with lysis of sensitized leukocytes Because the reaction in vitro gradually increases with the strength of the solution we have regarded it as a toxic rather than an allergic reaction Previous attempts to obtain proof of an allergic reaction as a cause of hyperpyrexia have failed Montegriffo (8) performed a patch test and gave intradermal injections of methyldopa at increasing strengths (0 1–1 0%) of the L isomer but the results were negative as was the Prausnitz Kustner reaction He further attempted to show the presence of circulating antibodies by using simple flocculation and gel diffusion precipitation techniques No precipitations were demonstrated at any time however In spite of the negative tests Montegriffo (8) regards the hyperpyrexia observed in his cases as an allergic hypersensitivity reaction mediated by an antigen antibody reaction although circulating antibodies could not be detected He stresses however that these are often extremely difficult to demonstrate and that the skin tests rarely detect drug sensitivity In spite of these negative findings Montegriffo is inclined to regard the hyperpyretic reaction as an allergic one mainly because of the mechanism behind any kind of drug sensitivity and because untoward reactions of this type have usually been regarded as allergic

Our findings correspond to those of Montegriffo i e they give no objective evidence of an allergic reaction However they suggest the possibility of toxic leukocytolysis as the causative mechanism underlying the hyperpyrexia

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IN VITRO DETECTION OF TUBERCULIN HYPERSENSITIVITY IN MAN

Specific Migration Inhibition of White Blood Cells from Tuberculin positive Persons

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Abst act The influence of purified protein derivative of tubercul n (PPD) upon the in vitro migration of peripheral leucocytes from persons with positive and negative intra cutaneous tuberculin reactions has been investigated An inhibition is seen with leucocytes from Mantoux positive persons whereas no inhibition is seen with leucocytes from Mantoux negative persons The migration indices are well correlated to the degree of the intracutaneous reactions in mm

Rich and Lewis (8 9) were the first to prove that cellular (delayed type) hypersensitivity in experi mental animals can be demonstrated in vitro by examining the migration of immunocompetent cells The migration is inhibited specifically by antigen to which the animals exhibit a cellular hypersensitivity

Numerous animal experiments carried out more recently have shown that the inhibition of migra tion by antigen is a good parameter in vitro for cellular hypersensitivity Apart from tuberculin a number of other antigens have been employed

Only few studies of tuberculin hypersensitivity in man are available assessed on the basis of in hibition of cell migration by tuberculin

It was a characteristic of the early studies that inhibition of migration was d monstrated in cases of active tuberculosis whereas on rare occasions only inhibition was found in non tuberculous per sons with a positive cutaneous tuberculin reaction (? 6 7) In the cases where inhibition was demon strated it was not related to the intensity of the intracutaneous tuberculin reaction

Employing the capillary tube technique described by George and Vaughan (5) and modified by David et al (3) Thor (13 14) was recently able to demonstrate inhibition of cell migration in non tuberculous persons with a positive intra cutaneous tuberculin reaction However he did not mention any possible relationship between the degree of the inhibition of migration and the intensity of the intracutaneous tuberculin reaction

In these studies either human lymph node cells cultivated for three days before application in migration cultures were used (13) or peritoneal exudate cells from guinea pigs to which were added supernatants from cultures of human lym phocytes from the peripheral blood incubated for 24 hours with tuberculin (14)

The method is found to be specific and very sensitive but complicated and time-consuming

Søborg and Bendixen (10) showed that the capillary tube technique using human peripheral leucocytes is well suited for the demonstration of cellular hypersensitivity Furthermore in a study of the brucella hypersensitivity it was proved that the degree of the inhibition of cell migration is related to the intensity of the intracutaneous reaction (11)

Compared with the method employed by Thor there are the advantages that the above technique does not require much time no experimental ani mals are needed and the test can be carried out using peripheral blood

The object of the present study was to examine whether the method is suited also for the examina tion of tuberculin hypersensitivity

MATERIAL AND METHODS

The tuberculin positive group comprised 38 persons with a pos t ve intracutaneous tuberculin reaction. Thirty two had a pos tive Mantoux I s x had a positive Mantoux II

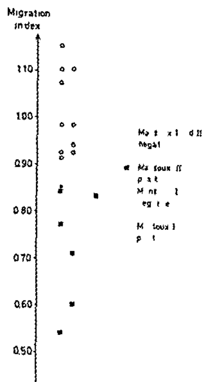


Fig 1 Migration indices of Mantoux positive and Mantoux negative persons

t a negative Mantoux I None of the persons presented ptoms of active tuberculous infection Some of them had a BCG vaccination others were spontaneously uberculin positive

The tuberculin negative group comprised 12 persons in whom both Mantoux I and II tests were negative

Patients with diseases which might influence the cellular hypersensitivity and patients being treated with glucocorticoids or cytostatics were not included in the study

The *leucocyte migration test (LMT)* was carried out employing the method described in further detail by Søborg and Bendixen (10) Leucocytes from peripheral blood were washed thoroughly and aspirated into capillary tubes which were placed in 1 ml culture chambers containing a culture medium. To half of the culture chambers 100 μl of a tuberculin solution were added containing 100 μg of purified protein derivative (PPD) (placed at our disposal by M Magnusson the Tuberculin Department of the State Serum Institute Copenhagen) To the other half of the chambers were added 100 μl of the phosphate-buffered solution which is used as a solvent for PPD

The cell migration areas after 24 hours were measured The average area in antigen-containing cultures divided by the average area in control cultures is termed the migration index (MI) MI is a measure of the antigen dependent inhibition. The stronger the inhibition, the lower the MI

The Mantoux tests were carried out by intracutaneous injection of 0 1 ml of tuberculin solution The solution used in Mantoux I contains 1 T U = 1/50 000 mg PPD per 0 1 ml The solution used in Mantoux II contains 10 T U = 1/5000 mg PPD per 0 1 ml The skin reaction is read after 72 hours The reaction is recorded as positive when the induration is 5 mm or more

LMT was always carried out before examination of the intracutaneous tuberculin reaction

RESULTS

Fig 1 shows the distribution of the migration indices in 38 persons with positive intracutaneous tuberculin reaction (32 positive after Mantoux I six positive after Mantoux II but negative after Mantoux I) and 12 persons with negative cutaneous tuberculin reaction after both Mantoux I and II

The mean value of the MI for the tuberculin positive persons is 0 74 with the standard deviation (S D) 0 13

The mean value for the tuberculin negative persons is 1 01 with S D 0 08 The two groups are significantly different ($p < 0.001$)

The lower limit for the normal range of the tuberculin negative persons is 0 85 (mean value -2 S D) As appears from the figure there is some overlapping between the two groups so that the MI for some of the tuberculin positive is in the lower half of the tuberculin negative normal range

Fig 2 shows the relationship between the size in mm of Mantoux I and the MI in both tuber

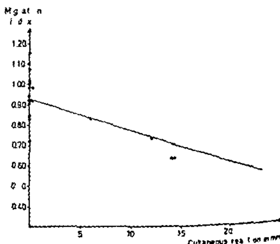


Fig 2 Thirty-eight Mantoux positive and twelve Mantoux negative persons Correlation of migration index and intracutaneous reaction

culin positive and tuberculin negative persons There is a good correlation between the intracutaneous reaction and the MI ($r = -0.61$ $p < 0.001$)

DISCUSSION

The present results seem to indicate that a tuberculin-dependent inhibition of cell migration of peripheral leucocytes is a good parameter in vitro for tuberculin hypersensitivity assessed from the intracutaneous tuberculin reaction However no inhibition was observed in about 15% of the tuberculin positive persons which seems to indicate that the sensitivity of the test is not quite satisfactory On the other hand in none of the tuberculin negative persons did the MI fall below 0 91 An MI below 0 85 (lower calculated limit of tuberculin negative persons) therefore will show with certainty that the person concerned is tuberculin positive On the other hand an MI above 0 85 can be seen in both tuberculin positive and tuberculin negative persons

In the present study an antigen concentration of 100 μg of PPD per ml was used It is possible that a better differentiation between tuberculin negative and tuberculin positive persons could be obtained by increasing the tuberculin concentration but this would involve the risk of a non specific toxic effect of the tuberculin On the other hand the antigen concentration employed is strong enough to ensure good agreement with the conditions in vivo as proved by the positive correlation between the intracutaneous test measured in mm and the inhibition of cell migration

In the early in vitro studies of human tuberculin hypersensitivity (2 6 7) tuberculin concentrations of the same order of magnitude were employed as those used in the present study Notwithstanding a concentration of 100 micrograms of tuberculin per ml O Neill and Favour (7) were not able to demonstrate any inhibition of the leucocyte migration in 11 out of 21 patients with tuberculosis Hall and Scherago (6) with a similar tuberculin concentration found that the cell migration in patients with active tuberculosis was distinctly inhibited in only 67% of the cases Patients with inactive tuberculosis and healthy persons with positive intracutaneous tuberculin reaction showed inhibition in only 21% and 9 of the cases respectively

Thor (13 14) used a tuberculin concentration of 10 μg per ml This was the case both in the experiments when he used lymph node cells and in the experiments when he used peritoneal exudate cells from guinea pigs to which were added supernatants from cultures of human peripheral lymphocytes cultivated for 24 hours with PPD Employing both experimental methods migration indices of the order of magnitude 0 20–0 30 were obtained

The difference between the necessary tuberculin concentration in the present study and in Thor s studies and the difference in the degree of the inhibition of migration are presumably caused by the employment of different cell systems

The influence of the composition of the cell population was first demonstrated by David et al (4) who showed that the migration of sensitive lymphocytes separated from lymph node cells was not inhibited by addition of the specific antigen whereas this produced a pronounced inhibition when added to a mixture of sensitive lymphocytes and macrophages Bloom and Bennet (1) showed that the migration of macrophages separated from peritoneal exudate cells from hypersensitive animals was also not inhibited by the antigen concerned The conclusion of these studies was that two types of cell were required for producing inhibition of cell migration by antigen One cell type should be sensitive lymphocytes the other macrophages which need not of necessity originate from a hypersensitive organism

In experiments with peripheral human leucocytes Søborg (12) showed that sensitive lymphocytes separated from the blood could also not be inhibited by addition of antigen but that the presence of granulocytes and monocytes was necessary for the inhibition of migration

In the first studies carried out by Thor (13) the approximate composition of the cell population after cultivation of the lymph node cells for three days was macrophages 25 % and lymphocyte like cells 65% the remaining cells being non-classifiable In his most recent studies (14) the cell population is peritoneal exudate cells from guinea pigs mainly macrophages but also some lymphocyte like cells

It remains to be clarified how macrophages and a mixture of granulocytes and monocytes respectively contribute to an inhibition of the migration of the entire cell population The difference be

tween the results obtained by Thor and by us however might be explained by the fact that macrophages are more effective than a mixture of granulocytes and monocytes in mediating the inhibition of migration of the total cell population

ACKNOWLEDGEMENTS

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Acta med scand Vol 186 pp 231–234 1969

CENTRAL HEMODYNAMIC EFFECTS OF TERODILINE IN PATIENTS WITH CORONARY HEART DISEASE

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Abst act The hemodynamic response to terodiline has been tested on ten patients with severe coronary heart disease The drug had no significant effects on the central hemodynamics at rest. During exercise however terodiline reduced the pulmonary capillary venous pressure at an increased or unchanged calculated external cardiac work in e ght of the patients No pain or reduced pain during exercise was noted by four of these patients

Terodiline or Bicor® (1 methyl 3 3 diphenyl 1 N tert butyl propylamine hydrochloride) possesses a slight local anesthetic effect and also has anti cholinergic spasmolytic and antihistaminic prop erties It has no effect on adrenergic α or β receptors In animal experiments using different pharmacological standard techniques terodilin produces marked increases in coronary blood flow through relaxation of smooth muscle cells in the vas ular wall resulting in decreased coronary vascular re istance (4) (Terodiline (Bicor®) from AB Recip Stockholm)

It has therefore been suggested that this drug could be used in the treatment of coronary insuffi ency (angina pectoris) (5)

In some clinical trials it has been demonstrated at terodiline has beneficial effects on patients h angina pectoris due to coronary heart disease Thus Wibell (6) demonstrated favorable effects nitroglycerin consumption and pain incidence a double blind cross-over study on 16 patients th coronary artery disease in which terodiline matched against a long acting nitroglycerin eparation Graf (1) and Astrand (7) found some with improved exercise tolerance and reduc on of ischemic ST-changes during or after exer cise in a small group of patients studied with exercise electrocardiography one hour before and 0 minutes after an oral dose of 25 mg terodiline

The clinically recommended dose of terodiline is 25 mg t.i d per os With this dosage blood levels of terodiline equal to those one hour after an intravenous injection of 75–100 mg are ex pected within 48 hours of therapy

The aim of the present investigation was to study the hemodynamic effects of intravenously injected terodiline by means of cardiac catheteriza tion The study was made on patients with angina pectoris produced by light physical exercise

MATERIAL AND METHODS

Ten males between 4_ and 64 years of age all with typical clinical electrocardiographic and coronary angiographic signs of coronary heart disease were stud ed The studies were performed as part of pre or post operative evaluation of their coronary and myocardial status before or one year after surgery aimed at im proving their myocardial blood supply This evaluation included a careful med cal history exercise tol rance tests with ECG recordings and coronary angiography None of the patients had signs of congestive heart failure or rheumatic heart disease and all had a sinus rhythm at the time of the study

The studies were made in the morning when the pa tients were in the postabsorptive basal state Right heart catheterization was performed with a double lumen cath eter and the brachial artery was catheterized using per cutaneous techn que Cardiac outputs were measured by the indicator dilution technique using bromsulfalein in jected via the right heart catheter into the pulmonary artery Inductance manometers and a four-channel m ngograph (AB Elema, Stockholm, Sweden) were used for registration and recording of pressures. The pat ents were studied in the supine position Exercise tests were per formed on a bicycle ergometer (AB Elema)

Tensions of oxygen and carbon d oxide and pH in blood samples were measured with conventional electrodes (Instrumentation Laboratories Inc) and oxygen contents were calculated us ng the Se eringhaus nomogram (3)

Table I *Hemodynamic data and differences at rest and during e[illegible]se in 10 patients with coronary insufficiency before (B) and one hour after (A) intravenous administration of terodiline with statistical analysis and probability (P) of obtained results*

Case no	Dose	Experimental condition	Heart rate (beats/min)		Stroke volume (ml)		Q (l/min)		a-v O_2 diff (ml/100 ml)		$\bar{P}_{PCV}$ (mm Hg)		$\bar{P}_{PA}$ (mm Hg)		P_{BA} Systolic (mm Hg)		P_{BA} Diastolic (mm Hg)		$\bar{P}_{BA}$ (mm Hg)		Pulm vasc resistance		Syst vasc resistance		LVSW (P)	
			B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
1	50 mg terodiline	Rest	60	60	97	75	5 8	4 5	43	48	10	1	13		166		121		136		0 5		23			
		Diff	±0		?2		1 3		+5		−9															
		300 kpm	78	82	125	100	9 7	8 2	90	89	16	6	31						108	100	1 5		11	12	156	128
		Diff	+4		−25		1 5		−1		−10								−8				+1		−8	
2	50 mg terodiline	Rest	6?	64	81	83	5 0	5 3	32	34	10	12	16	[illegible]0	143	145	87	90	102	113	1 2	1 5	20 4	21 3	101	114
		Diff	+2		+2		+0 3		+2		+2		+4		+2		+3		+11		+0 3		+0 9		+13	
		150 kpm	98	90	68	71	6 7	6 4	61	61	45	25		35	148	163	103	100	119	127		1 6	17 8	19 8	68	98
		Diff	−8		+3		0 3		±0		−20				+15		−3		+8				+2 0		+30	
3	50 mg terodiline	Rest	68	74	94	103	6 4	7 7	41	45	8	7	12	16	149	161	81	90	102	116	0 6	1 2	15 9	15 1	120	176
		Diff	+6		+9		+1 3		+4		−1		+4		+12		+9		+14		+0 6		−0 8		+6	
		250 kpm	94	96	103	98	9 7	9 4	80	93	25	17	33	32	172	174	95	94	132	130	0 8	1 6	13 6	13 8	98	103
		Diff	+2		5		0 3		+13		−8		−1		+2		−1		−2		+0 8		+0 2		+5	
4	75 mg terodiline	Rest	66	70	96	77	6 3	5 4	49	44	5	8	12	13	154	144	86	82	116	105	1 1	0 9	18 4	19 4	145	102
		Diff	+4		19		−0 9		−5		+3		+1		−10		−4		−11		−0 2		+1 0		−43	
		450 kpm	102	11?	128	99	13 0	11 0	89	93	19	14	30	23	168	174	92	93	129	131	0 8	0 8	9 9	11 9	191	158
		Diff	+10		29		2 0		+4		−5		−7		+6		+1		+2		±0		+2 0		−33	
5	75 mg terodiline	Rest	80	80	108	118	8 7	9 4	28	38	3	3	7	9	173	161	82	77	107	110	0 5	0 6	12 3	11 7	153	172
		Diff	±0		+10		+0 7		+10		±0		+2		−12		−5		+3		+0 1		−0 6		+19	
		?50 kpm	106	104	115	1 9	12 2	13 4	68	75	12	11	23	21	200	191	92	95	147	136	0 9	0 7	12 0	10 1	211	219
		Diff	[illegible]		+14		+1?		+7		−1		−2		−9		+3		−11		−0 2		−1 9		+8	
6	50 mg terodiline	Rest	66	80	68	66	4 5	5 3	39	36	5	9	9	12	75	100	30	65	48	82	0 9	0 6	10 7	15 5	40	66
		Diff	+14		−2		+0 8		−3		+4		+3		+25		+35		+34		−0 3		+4 8		+26	
		600 kpm	140	134	77	75	10 7	10 1	77	80	20	16	37	31	185	189	90	100	135	140	1 6	1 5	12 6	13 9	120	126
		Diff	−6		−2		−0 6		+3		−4		−6		+4		+10		+5		−0 1		+1 3		+6	
7	50 mg terodiline	Rest	58	74	9	79	5 3	5 9	39	38	10	7	19	17	160	159	73	82	97	107	1 7	1 7	18 3	18 1	109	107
		Diff	+16		−13		+0 6		−1		−3		−2		−1		+9		+10		±0		−0 2		−2	
		?00 kpm	110	102	59	74	6 4	7 5	95	75	40	15	54	35	193	183	104	90	115	133	2 2	2 7	18 0	17 7	60	119
		Diff	−8		+15		+1 1		−20		−25		−19		−10		−14		+18		+0 5		−0 3		+59	
8	100 mg terodiline	Rest	96	96	68	59	6 5	5 7	44	52	1	4	10	10	170	172	117	125	140	142	1 4	1 0	21 5	?4 9	1 9	111
		Diff	±0		9		−0 8		+8		+3		±0		+[illegible]		+8		+2		−0 4		+3 4		−18	
		100 kpm	96	100	73	84	7 0	8 4	75	87	15	5	24	[illegible]0					140	138	1 3	1 8	[illegible]0 0	16 4	124	152
		Diff	+4		+11		+1 4		+12		−10		−4						−2		+0 5		−3 6		+[illegible]8	
9	50 mg t[illegible]odil[illegible]n	Re[illegible]t	54	56	113	102	6 1	5 7	40	43	9	5	11	12	133	136	72	88	91	119	0 3	1 2	14 9	[illegible]0 9	1 6	158
		D[illegible]ff	+2		−11		−0 4		13		−4		+1		+[illegible]3		+16		+[illegible]8		+0 9		+6 0		+3[illegible]	
		[illegible]00 kpm	96	100	87	97	8 5	9 7	93	112	4	0	33	31	164	167	85	87	118	1[illegible]0	1 1	1 1	14 2	12 4	111	132

t n d ll	D ff 300 kpm D ff												
		+ 6 116 1 4 + 8	− 12 66 85 + 19	± 0 7 7 10 5 + 8	+ 4 9 84 − 8	± 0 1 13 − 8	− 2 9 21 − 8	− 10 0 35 + 15	+ 9 1 3 111 + 8	− 13 153 148 − 5	− 0 6 1 0 0 7 − 0 3	− 6 19 9 14 1 − 5 8	− 31 118 156 + 38
R t	n	10	10	10	10	10	9	9	9	10	9	9	9
	$\bar{d}$	7	− 6 7	0 0	7	− 0 5	1 2	3 4	8 9	8 7	0 0	1 3	0 2
	S D	8 8	11 1	0 9	4 7	3 9	2 3	13 9	11 9	15 8	0 5	2 8	26
	S E	2 8	3 5	0 3	1 5	1 2	0 8	4 4	4 0	5 3	0 2	0 9	8 7
	P	< 0 05	< 0 05	0 05	> 0 05	> 0 05	> 0 05	> 0 05	> 0 05	> 0 05	> 0 05	> 0 05	> 0 05
Exe cise	n	10	10	10	10	10	8	8	8	10	8	10	10
	$\bar{d}$	0 8	1 1	0 3	7	− 9 5	− 6 1	3 3	0 8	0 7	0 2	0 7	13 4
	S D	6 5	16 7	1 5	10 9	7 5	5 8	9 4	7 4	8 4	0 4	2 6	28 4
	S E	2 1	5 3	0 5	3 5	2 4	2 1	3 3	2 6	2 7	0 1	0 8	9 0
	P	> 0 05	> 0 05	0 05	> 0 05	< 0 01	< 0 05	> 0 05	> 0 05	> 0 05	> 0 05	> 0 05	> 0 05

Vascular resi tances were calculated according to the formulae

pulmonary vascular resistance $PVR = \bar{P}_{PA} - \bar{P}_{PCV}$ Q
systemic vascular resistance $SVR = \bar{P}_{BA}/Q$

expressed in arbitrary units Left ventricular stroke work was calculated according to the formula

$$LVSW = \frac{SV(\bar{P}_{BA} - \bar{P}_{PCV}) \times 13\ 6}{1000}$$

expressed in ponds per beat

Measurements were made with the patients at rest and during an exercise test with a level of work predeter minated to provoke supportable an ina pectoris. A few minutes after the exercise study was completed the tip of the double lumen catheter was withdrawn from "wedge position so that it became located in the main pulmonary artery An intravenous injection during five minutes of a solution containing 50–100 mg of terodiline was given The patients were then allowed to rest for approximately one hour The tip of the catheter was then relocated in the wedge position Rest and exercise studies at the initial work load were now repeated No untoward subjective effects of the drug were noted either during the rest periods or during the exercise studies

RESULTS

The results of the hemodynamic studies are pre sented in Table I With the exception of a probably significant increase in heart rate ($\bar{d} = +7$ beats/min) and a decrease in stroke volume ($\bar{d} = -7$ ml) there were no changes induced by terodi line on the circulatory variables at rest.

The effects of terodoline on the hemodynamics during exercise were somewhat more pronounced In all cases there was a reduction in the pul monary artery mean pressure ($\bar{d} = -7$ mm Hg) ($P < 0\ 05$) and a similar reduction of the filling pressure of the left ventricle (estimated from the pulmonary capillary venous pressure) of 10 mm Hg ($P < 0\ 01$) was also observed The individual reductions were in some cases quite pronounced and not associated with any marked reductions in cardiac output, mean arterial blood pressure or systemic vascular resistance

No significant changes were observed in cardiac output a–v O -difference brachial artery pres sures vascular resistances or calculated left ven tricular stroke work during exercise As can be seen in Table I there were inter individual varia tions in the effect of terodiline on these variables

Four patients (nos 2 4 7 8) claimed to ex perience no pain or definitely less pain during exercise while the remainder noted no difference in pain after the drug had been administered

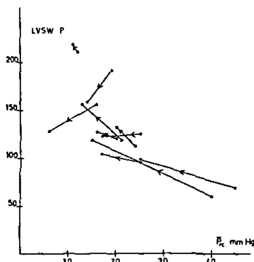


Fig 1 Changes in pulmonary capillary venous pressures ($\bar{P}_{PC}$) and left ventricular stroke work (LVSW) during exercise induced by terodiline in 10 patients with coronary insufficiency

The simultaneous changes in capillary venous pressure and left ventricular stroke work during exercise induced by terodiline are illustrated in Fig 1 This comparison demonstrates that in eight out of ten patients the estimated left ventricular stroke work during exercise was unchanged or increased at a lower left ventricular filling pres after administration of terodiline

DISCUSSION

The results of this small hemodynamic study would indicate that acutely intravenously ad ministered terodiline has effects upon the central hemodynamics during exercise in patients with coronary heart disease The most striking effect is the lowering of the PCV pressure which indi cates improvement of left ventricular failure This effect is definitely different from that encountered after the administration of a placebo in a similar situation (2) The shift to the left and upwards in the relation between filling pressure of the left ventricle (pulmonary capillary venous pressure) and left ventricular stroke work in the majority of the cases indicates improved left ventricular function during exercise This effect is similar to that induced by digitalis administered in a similar fashion (2)

The myocardial function *in patients with* cor onary heart disease may be altered by changes in myocardial contractility oxygen utilization oxy gen demand or oxygen supply It is not yet fully clear which of these theoretical modes of action applies in the case of terodiline although the results of animal experiments indicate that it should increase myocardial blood supply

Our findings demonstrate that after administra tion of terodiline there were no untoward subjec tive effects and there was a definite subjective *improvement in four cases Objectively measured* improvement was noted in eight out of ten pa *tients some of them very striking On the basis of* these results obtained with intravenous administra tion we believe that this drug should be tried as an adjunctive also in oral long term therapy of angina pectoris

ACKNOWLEDGEMENTS

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LACTOSE MALABSORPTION IN GREENLAND ESKIMOS

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Abstract The incidence of lactose malabsorption in 3? Greenland Eskimos has been studied by means of sugar tolerance tests and in nine cases determination of di dase activity in jejunal biopsies. Twenty three (7?%) had lactose malabsorption If s ven Eskimos with a Danish ancestor are excluded the incidence of lactose malabsorption is 88% (22 of 25)

Lactose malabsorption seems to be a distinctive racial feature in Eskimos Clinical signs of lactose malabsorption were rare but so was milk consumption in general However clinical reactions to lactose ingestion during a lactose tolerance test were less pronounced than in lactase deficient white patients

Man s ability to retain small intestinal lactase activity for the rest of life after the weaning period is extraordinary within the class of mammals There are however exceptions since racial occurrence of lactase deficiency has recently been described in various areas of the world It may reflect the fact that in man s history domestication of cattle and pastoralism with easy access to milk after weaning were introduced at different times in different parts of the world (13)

The incidence of lactose malabsorption in Eskimos has so far not been studied If a connexion exists between the evolution of husbandry thousands of years ago and adult intestinal lactase activity one might expect lactase deficiency to be frequent in Eskimos Not until the last few decades have dairy products been obtainable in the polar deserts

We here report studies of lactose absorption in a limited number of Greenland Eskimos Our results suggest that the incidence of lactose malabsorption among Eskimos is much higher than in Western Europe and the United States

MATERIAL

Thirty two Greenland Eskimos were studied They were all admitted from Greenland to this hospital because of diseases which, in the great majority were unrelated to the gastrointestinal tract None had malabsorption or any other sign of small intestinal disease Their mean a e was 33 years (observed range 13 to 75 years) Sixteen were females 16 men Seven patients knew of a European Danish ancestor th remaining 25 considered themsel es pure" Eskimos

METHODS

Sugar tolerance tests A lactose tolerance test (LTT) was performed in each case Blood glucose was determined in capillary blood by a glucose oxidase method before and 15 30 60 90 and 120 min after oral administration of 100 g lactose dissolved in water (8) Similarly a glucose galactose tolerance test with 50 g glucose +50 g galactose was performed in 21 cases and a sucrose tolerance test with 100 g sucrose in 17 cases.

Small intestinal disaccharidase activity In nine cases jejunal biopsies were obtained at or up to 40 cm distal to the ligament of Treitz. Lactase sucrase and maltase activity were determined by Dahlqvist s one step method (11) All biopsies were normal on stereomicroscopy and routine microscopy

RESULTS

Tolerance tests A flat blood sugar curve (i e less than 25 mg increase of glucose per 100 ml blood (8)) in the LTT was found in 22 of 25 Eskimos without Danish ancestors but in only one of seven who knew of a Danish ancestor (Fig 1) A normal rise of blood sugar followed ingestion of glucose galactose and of sucrose in all examined cases

Six of 23 Greenlanders with flat blood sugar curves in the LTT displayed no symptoms during

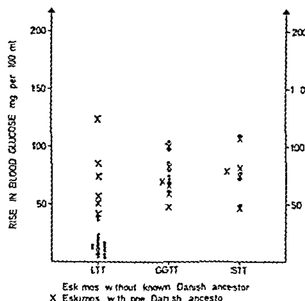


Fig 1 Maximum blood sugar rise in mg per 100 ml during lactose tolerance test (LTT) glucose galactose tolerance test (GGTT) and sucrose tolerance test (STT) in 32 Greenland Eskimos

the test The remaining 17 had diarrhea and/or abdominal discomfort The other tolerance tests produced no symptoms

Jejunal mucosal lactase activity was abnormally in eight cases from 0 to 3 international units r g protein (normal figure >9) The LTT was abnormal in seven In the eighth who had diarrhea both after the LTT and when he drank milk the blood sugar rose by 30 mg per 100 ml

A ninth patient had normal jejunal lactase activity His LTT was normal too

Mucosal sucrase and maltase activity was normal in every case

Symptoms of milk intolerance Nine patients complained of intermittent diarrhea Eight of them had lactose malabsorption All drank milk daily Only two had observed that diarrhea followed milk intake and three had noticed abdominal cramps bloating and flatulence after milk Nevertheless they continued to drink milk

Sixteen patients did not drink milk daily Fourteen of them turned out to have lactose malabsorption

Among 15 patients who drank milk daily lactose malabsorption was present in only nine

Information on milk drinking habits is missing in one case because of a temporary lack of an interpreter

DISCUSSION

The incidence of specific lactose malabsorption (i e lactose malabsorption unassociated with other gastrointestinal diseases or with malabsorption of monosaccharides or other disaccharides) in white people in USA and Western Europe seems to be about 6% (1 6 7 12) although in a few series lactose malabsorption occurred in 16 to 18% (9 14) In contrast a much higher incidence has been found in other races e g 70 and 73% in 20 respectively 41 American negroes (1 4) 88% in 17 Greek cypriots (10) 89% in an African Bantu tribe (3) 100% of 11 Orientals (2) and 87% of 15 Chinese students (5)

In the present study lactose malabsorption was present in 23 of 32 Greenland Eskimos (72%) If only pure Eskimos are considered 22 of 25 (88%) had lactose malabsorption The results indicate that lactose malabsorption is a distinctive feature in the Eskimo race Since the Greenland Eskimos are supposed to be descendants of Canadian Eskimos who have been emigrants to Greenland over the last 4000 years it is to be expected that a similar high incidence of lactose malabsorption prevails among Eskimos in Northern America

The clinical significance of lactose malabsorption in Eskimos is difficult to assess for the moment It will probably grow concurrently with an easier access to milk and milk products at distant villages along the 39 000 km coastline of Greenland However it was remarkable to note that the clinical reaction to lactose during the LTT was absent or weak in many Eskimos with lactose malabsorption Thus of 23 cases with lactose malabsorption only 11 had diarrhea and six had no complaints whatsoever This is in sharp contrast to the severe reactions in the great majority of lactase-deficient European patients (7)

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Ac a med scand Vol 186 pp 239-240 1969

EFFECT OF ANTI LEUKEMIC DRUGS ON CELL CYCLE OF HUMAN LEUKEMIC BLAST CELLS IN VIVO

PRELIMINARY REPORT

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It has become clear from clinical trials of chemotherapy in acute leukemia that the chance of inducing remission is less when a single agent is employed than when drugs are used in combination moreover results depend on how the agents used are applied (2) At the same time a general picture of the kinetics of leukemic blast cells in man is emerging (4) Adaptation of chemotherapy to the kinetics of leukemic cells may further improve therapeutic results If it can be defined where and for how long leukemic cells are inhibited in their progression through the cell cycle by various anti leukemic drugs this may provide a rational basis for selecting the optimal sequence and spacing of drugs Ultimately kinetic differences between leukemic and normal cells might be exploited to obtain more selective cytotoxicity Preliminary observations on the cell kinetic effects of prednisone and methotrexate are presented in this report

METHODS

After drug administration serial bone marrow samples are obtained from the patient and incubated with H thymidine (H^3TDR) for one hour (3) The percentage of H^3TDR labeled cells (labeling index) = I_L is determined from autoradiographs Measurements of DNA content of individual cell nuclei and their H^3TDR incorporation are done as follows (1) cells identified in Giemsa-stained preparations are plotted on photographic maps the stain is removed and, after Feulgen staining nucl ar DNA is measured with the Zeiss UMSP I instrument The slides are processed for autoradiography the cells relocated and their H^3TDR labeling determined

RESULTS

1 *Effect of prednisone*

A 21 year-old man with untreated acute lymphoblastic leukemia was given 200 mg of prednisone in one oral dose and then 25 mg every 6 hours Immediately before therapy the H^3TDR I_L of bone marrow lymphoblasts was 27 6 Sixteen hours later it had dropped to 5 8 % and subsequently was reduced even further (Fig 1 (*a*)) The clinical effect was equally impressive and full remission was obtained by treatment with prednisone and vincristine When relapse took place the patient was put on the same prednisone regimen The H^3TDR I_L of marrow lymphoblasts fell from 27 0 % before prednisone to 6 6 % 24 hours after the first prednisone dose (Fig 1 (*b*)) I_L of myelocytes was not reduced The fall in I_L could not be ascribed to variable admixtures of circulating blast cells which have lower I_L than marrow blasts (3 4) Fig 2 shows the DNA measurements of the blast cells Before prednisone 25% of the cells had a DNA content between 2 n and 4 n i e in the range of DNA replication Twenty four hours later 3 % of the cells were in this range This is in good agreement with the H^3TDR data and excludes the possibility that the drop in H^3TDR I_L was due to a prednisone effect preventing DNA synthesizing cells from utilizing exogenous thymidine It is seen from Fig 2 that cells with a DNA content between 2 n and 4 n but not incorporating H^3TDR are rare

Prednisone may lyse non proliferating leukemic lymphoblasts but the data show that prednisone at least in this patient acted preferentially on proliferating lymphoblasts This raises the question whether it is rational to administer primarily DNA synthesis blocking agents like methotrexate at the time of maximal prednisone effect on the cell cycle However this may depend on whether or not cellular methotrexate uptake is cell cycle

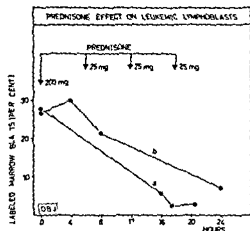


Fig 1 H^3TDR I_L of bone marrow lymphoblasts in a patient with acute lymphoblastic leukemia after start of prednisone treatment *a* at diagnosis *b* at first relapse

phase dependent This is being studied in this laboratory

2 Methotrexate effect

Four patients with acute myeloblastic leukemia have been studied after a single intravenous dose of 15–20 mg of methotrexate In all cases methotrexate was followed by rapid increases in the H^3TDR I_L of marrow blasts which reached twice the pre treatment values within 8 hours increases above the pre treatment level have been observed up to 24 hours The results cannot be explained by variable contamination of circulating blasts nor by diurnal variations of I_L known to be small or absent (5 6) If methotrexate prevented progression through the DNA synthesis phase (S-phase) without affecting the normal entry rate into S these data would suggest a DNA-synthesis time of approximately 8 hours However most in vivo studies indicate a considerably longer S-phase of leukemic myeloblasts (4) This raises the possibility that methotrexate may trigger off an acute extra recruitment of cells in pre S-phase (G_1) into S-phase One would then expect a piling up of labeled cells in early S during the first hours after methotrexate Our microspectrophotometric data as yet incomplete show such a tendency it is doubtful however whether this will quantitatively account for the rise in the H^3TDR I_L

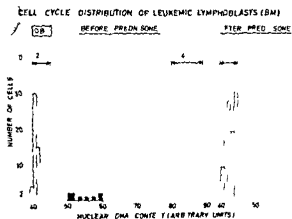


Fig 2 DNA content of leukemic lymphoblasts in bone marrow (same patient as in Fig 1) at time of first relapse *n* and *4n* are the diploid (granulocyte value) and tetraploid DNA values, respectively 24 hours after initiation of prednisone cells with > *n* DNA have virtually disappeared The abscissa relating to the after prednisone cells has been cut since no cells with more than 48 arbitrary units of DNA were observed Solid bars indicate cells labeled with H TDR, open bars represent unlabeled cells

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CATECHOLAMINES AND METABOLISM OF HUMAN ADIPOSE TISSUE

I *Comparison between in vitro Effects of Noradrenaline Adrenaline and Theophylline on Lipolysis in Omental Adipose Tissue*

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Abstract Lipolysis in human omental tissue incubated in glucose-containing medium was determined by measuring glycerol release in the presence of different concentrations of noradrenaline and adrenaline Almost identical responses were obtained with the two catecholamines significant increases in the glycerol release were obtained when they were added at concentrations as small as 0.03×10^{-6} *M* and maximal lipolysis was produced at $0.3-3 \times 10^{-6}$ *M* Noradrenaline as well as 10^{-} *M* of theophylline induced increases in the glycerol release were well correlated with the basal glycerol release

Maximal glycerol release obtained with theophylline (10^{-} *M*) was almost constantly six to eight times the basal glycerol release whereas the lipolytic effect of noradrenaline was more variable and in no experiments exceeded significantly the theophylline effect. The results indicate that the formation of cyclic adenosine 3,5 monophosphate (3 5 AMP) or some process between the adrenergic receptor and the adenyl cyclase system is rate limiting for the lipolytic response to noradrenaline The data also suggest that variations in the lipolytic response to noradrenaline of adipose tissue removed from different subjects are principally caused by variations in the effect of noradrenaline on the formation of 3,5 AMP

The present material of 32 patients does not allow critical analysis of the possible influence of sex, age and bodyweight of the donors on the tissue response to noradrenaline However the data indicate that adipose tissue obtained from old subjects has less lipolytic response than such tissue from young subjects

The major role of the sympathetic nervous system in the control of lipid mobilization is well documented and has been reviewed by Havel (11) Wenke (26) and Himms Hagen (14) A number of hormones have adipokinetic action in the rat and other low mammals (23) In human subjects by contrast only catecholamines (5 8 12 19) and glucagon (6) have been shown to have the property of increasing acutely the mobilization of FFA from the fat depots in vivo It has been demonstrated by several authors (1 2 4 7 9 16 18 20) that noradrenaline and adrenaline increase the release of FFA and glycerol from human adipose tissue in vitro The activation of the catecholamine sensitive lipase demonstrable in human adipose tissue (1) is most likely mediated through the formation of adenosine 3 5 monophosphate (3 5 AMP) since theophylline in this tissue as in rat adipose tissue exerts a strong lipolytic effect (24) This is presumably due to inhibition of the phosphodiesterase activity and thereby prevention of the degradation of 3 5 AMP (3 22 25) Investigations of human adipose tissue have shown that the lipolytic response to noradrenaline as well as to adrenaline is rather variable (2 4)

The purpose of the present investigation was to further investigate the potencies of noradrenaline and adrenaline in human adipose tissue in vitro and to study the reason for this variable response to noradrenaline Future papers in this series of studies will deal with the effect of the catecholamines on the synthesis of triglycerides in human adipose tissue the association between the formation and the breakdown of tissue triglycerides and the effect of adrenergic blocking agents on these processes

MATERIAL AND METHODS

Omental adipose tissue was obtained from a total of 3 patients undergoing cholecystectomy None of these pa

Table I *Sex age and body weight distribution of subjects employed in this investigation*

Sex	Ideal body weight[a] ( )	Number of subjects 17–40 y	41–60 y	61–80 y
♂	81–100	2	4	6
	101–120	1	3	0
♀	81–100	4	2	3
	101–120	5	2	0

[a] According to the Tables computed by Metropolitan Life Insurance Co (17)

tients showed evidence of diabetes mellitus or other metabolic disorders Age sex and ideal weight (17) of the donors are presented in Table I From the initial material of 34 consecutive patients two were later rejected in order to include only those patients who weighed within 80 to 1 0 of the ideal body weight All patients were operated after overnight fasting After induction with ultra short acting barbiturate general anesthesia was maintained with halothane and nitrous oxide in combination with suxomethonium chloride The adipose tissue was removed at the start of the operation, placed into albumin containing buffer kept at 37 C and divided into pieces weighing about 50 mg each After 60 min of preincubation the tissue portions were transferred into Packard plastic vials containing 3 ml of Krebs Henseleit bicarbonate buffer (pH 7 4) with the addition of 3 g/100 ml of bovine serum lbumin (Armour Pharm Co Eastbourne England Lot M G 2770) and glucose (100 mg/100 ml) The ar albumin/FFA ratio was 1 3 About 200 mg of adipose tissue was incubated in each vessel All incubations were run in duplicate Aliquots of the medium were removed after two hours of incubation unless otherwise stated Glycerol in the medium was determined by the enzymatic procedure of Wieland (27) as modified by Larsen (15) Handling of tissue and incubation procedure have been described in detail previously (28) In vitro additions of one or two of the following agents were made to the medium adrenaline bitartrate and noradrenaline bitartrate (supplied as pure substances by Astra AB Sodertalje Sweden) and theophylline obtained commercially

RESULTS

Fig 1 shows that noradrenaline added at a concentration as small as $0\,03 \times 10^{-5}$ *M* (approximately 0 05 µg/ml) stimulated the glycerol release from omental adipose tissue incubated in the presence of glucose (1 mg/ml) This effect was statistically significant ($p < 0\,001$) In all but one experiment a tenfold increase in this noradrenaline concentration resulted in maximal stimulation

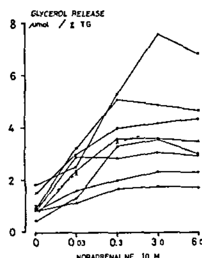


Fig 1 Omental adipose tissue (approx 200 mg) divided into sections of about 50 mg each was incubated for two hours in 3 ml of Krebs-Henseleit bicarbonate buffer (pH 7 4) containing 3 (w/v) of bovine serum albumin and 1 mg/ml of glucose Each point represents the mean of two incubations The dotted line indicates the mean glycerol release from adipose tissue obtained from eight patients during surgical operation

of the glycerol release Lipolysis was neither accelerated further nor reduced by the addition of concentrations as high as 3×10^{-5} *M* and 6×10^{-5} *M* In a single experiment adipose tissue showed marked lipolytic response to noradrenaline and the concentration of 3×10^{-5} *M* was required to give the maximal effect The average increment in glycerol release produced by noradrenaline in these experiments was a fourfold increase Fig 2

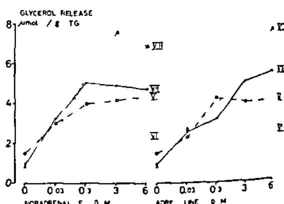


Fig 2 Comparison of the effects of noradrenaline and adrenaline on the glycerol release from omental adipose tissue in vitro The incubation procedure is given in Fig 1 Roman numerals refer to the serial numbers of the patients included in this investigation

illustrates four experiments in which adipose tissue of each donor was incubated in the presence of either noradrenaline or adrenaline added at four different concentrations to the incubation medium It was observed that the dose response curves for noradrenaline and adrenaline were rather similar and that the differences between the two agents were small and inconsistent

Two types of experiments were undertaken in order to study which factor(s) might be rate limiting for the lipolytic response to noradrenaline In the first series of experiments the rate of lipolysis was first studied as a function of time We observed that the basal as well as the noradrenaline induced glycerol release was linear throughout two hours of incubation From two typical experiments presented in Fig 3 it is apparent that 0 3 × 10^{-5} *M* and 6 × 10^{-5} *M* of noradrenaline produced almost identical increases in the glycerol release determined after 10 40 70 and 130 min of incubation In the second set of experiments the effect of adding a maximal dosage of noradrenaline (3 × 10 ⁻ *M*) to the incubation medium was compared with (*a*) the effect of a maximal dosage of theophylline (10⁻ *M*) and (*b*) the effect of both agents added together The concentration of theophylline selected was one which had been found in previous studies to produce maximal stimulation of glycerol release from human omental tissue These agents stimulated lipolysis by different mechanisms noradrenaline increases the for

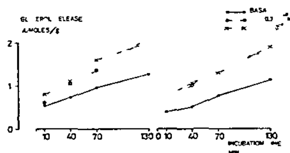


Fig 3 Comparison of the effect of different concentrations of noradrenaline on the glycerol release from omental adipose tissue incubated 10 40 70 and 130 min in Krebs-Henseleit bicarbonate buffer containing bovine serum albumin (3%) and glucose (1 mg ml) ● basal O---O NA 0.3 × 10^{-5} M ×---× NA 6 × 10^{-5} M

mation of 3 ,5 AMP (3 22) whereas theophylline inhibits the degradation of this nucleotide (25) The results of these studies (Table II) indicate that the responses to theophylline were of similar magnitude since the increase in glycerol release varied from six to eight times the basal release in nine out of ten experiments In five subjects (XI XXVIII XXIX XXXI XXXII) it was observed that theophylline was a significantly more potent lipolytic agent than noradrenaline whereas in the other five experiments no differences between the agents were found. In these latter patients the addition of theophylline to noradrenaline-containing medium resulted in less than 50% increase

Table II *Effects of noradrenaline theophylline and both agents on the glycerol release from omental adipose tissue in vitro*

Pat.	Glycerol release Basal μmoles	Noradrenaline μmoles[a]	Noradrenaline per cent[b]	Theophylline μmoles[a]	Theophylline per cent[b]	Noradrenaline – theophylline μmoles
X	0 75	5 91	788	5 98	797	7 4?
XI	0 53	1.51	285	3 82	721	5.27
XIV	0 84	4 56	543	4 00	476	4 6
XXVI	0 94	6 44	685	7 56	804	8 61
XXVII	1 27	6 69	5 7	7 76	611	9 66
XXVIII	1 05	3 56	339	7 60	724	9 59
XXIX	0 98	4 01	405	5 81	593	9 65
XXX	0 7?	5 17	718	5.3	7 6	6 6
XXXI	0 99	2.36	238	6 48	655	11 0
XXXII	0 56	2.63	470	3 99	713	6 44

[a] Calculated per g lipid weight ⁻ h
[b] Calculated as percentage basal l polysis. Incubation procedure same as in F g. 1
Noradrenal ne conc. 3 10^{-4} M theophyl ne conc 10 M Correlations 1) basal versus noradrenaline-induced glycerol release r = 0 50 p > 0 10 2) basal versus theophylline-induced glycerol release r = 0 84 p 0 005 3) basal versus noradrenaline- plus theophylline-induced glycerol release = 0 6 p < 0 0

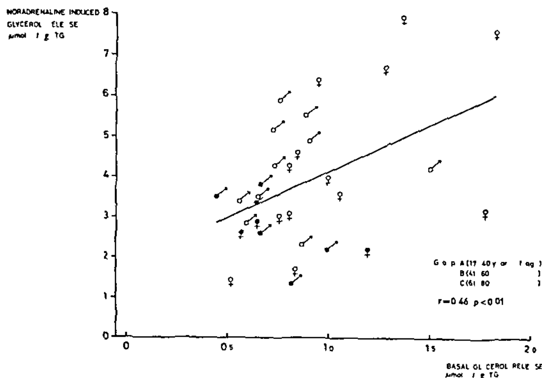


Fig 4 Relationship between the basal lipolysis and the glycerol release induced by noradrenaline ($3 \times 10^{-}$ *M*) in human omental adipose tissue Correlation coefficient $r = 0\,46$ $p < 0\,01$ Regression equation $Y = 2\,29\ X + 1\,86$

(mean 27 %) in the lipolysis produced by nor renaline alone By contrast theophylline had an most additive effect on the lipolysis of adipose from subjects showing a poor response to noradrenaline alone In these patients the lipolysis in adipose tissue was increased more than 140 % (mean 214 %) by theophylline Neither age nor sex of the donors seemed to be of importance for this difference in the response of adipose tissue to noradrenaline The lipolytic responses to theo phylline and noradrenaline plus theophylline were correlated with the basal lipolysis in this series of experiments The correlation coefficients are presented in Table II

The relationship between the effect of nor adrenaline on lipolysis and basal lipolysis ($r = 0\,46$ $p < 0\,01$) is illustrated in Fig 4 in which the results of tissue obtained from all 32 pa tients are included Since this small clinical material was rather heterogeneous with respect to age sex and possibly also to amount of body fat it is not possible to ascertain whether any of these factors was of significance for the response to noradrenaline However the results indicate that the lipolytic response to noradrenaline possibly decreased with increasing age of the donor Thus the glycerol release (µmoles/g lipid weight/2 h) from omental adipose tissue from patients in group C (61–80 years of age) was $2\,77 \pm 0\,76$ (mean $\pm$ standard deviation) and thus significant ly lower than from tissue of patients in group A (17–40 years of age) ($4\,99 \pm 2\,03$) $p < 0\,01$) and in group B (41–60 years of age) ($3\,74 \pm 1\,12$) ($p < 0\,05$) It is also evident from the figure that there were greater variations in the lipolytic re sponse of adipose tissue from young than from older subjects

DISCUSSION

Present data show that the lipolytic enzyme system of human omental tissue is remarkably sensitive to noradrenaline Noradrenaline added in as low a concentration as $0\,03 \times 10^{-5}$ *M* consistently in duced a marked increase in the glycerol release In most experiments noradrenaline produced maxi mal response when added in a concentration of $0\,3 \times 10^{-5}$ *M* and with 3×10 *M* maximal ef fect was obtained in all experiments presented and undertaken so far The lipolytic effect of nor

adrenaline and adrenaline in adipose tissue was of similar magnitude The less sustained increase in the plasma FFA level after adrenaline adminis tration compared with that after noradrenaline in vivo (10) is apparently due to the simultaneous effect of adrenaline on the glucose output from the liver and the subsequent inhibitory effect of blood glucose and/or insulin increase on lipolysis in adipose tissue

Rate limiting for the in vitro lipolytic effect of noradrenaline and adrenaline is apparently the formation of 3 5 AMP in human omental tissue or some step between the adrenergic receptor site and the adenyl cyclase system Neither the de gradation of the hormones during incubation nor the metabolic changes evoked in tissues can be rate limiting since during the whole incubation period the lipolytic response of adipose tissue was almost identical at low ($0\ 3 \times 10^{5}$ *M*) and high (6×10 *M*) concentrations of noradrenaline In addition it was observed in all experiments that the glycerol release from adipose tissue was fur ther enhanced either by theophylline alone or by theophylline plus noradrenaline This would ex clude the possibility that the capacity of the lipo-lytic enzyme system is rate limiting for the effect of noradrenaline

It was of interest to observe that two different patterns of lipolytic response to noradrenaline and theophylline existed in the adipose tissue The observation in five out of ten experiments that the effect of noradrenaline was significantly less than that of theophylline is best explained by the existence of some factor in these adipose tissue sections which decreases the ability of noradren aline to accelerate the rate of formation of 3 5 AMP In later experiments we have demonstrated that the lipolytic response to noradrenaline is stim ulated in the presence of an alpha adrenergic b ocking agent which suggests that the noradrena line effect on lipolysis is antagonized by alpha adren rgic re eptors (29) Our findings that the ophylline had an additive effect on the noradrena line induced lipolysis only in tissue showing poor response to noradrenaline cannot be explained until additional information about the relationship between 3 5 AMP and the lipase activity has been gained

The present data as well as later experiments have shown that the maximal glycerol release pro duced by theophylline is well correlated with the basal lipolysis and in nine out of ten experiments was six to eight times the basal release Assuming that during lipolysis no net consumption of 3 5 AMP takes place these results would indicate that the percentage degradation of 3 5 AMP pro-duced under basal conditions was rather constant for omental tissue removed from the present group of patients In contrast to other authors (4) we were able to detect a positive relationship between the basal glycerol release and the noradrenaline induced lipolysis in human omental tissue One possible explanation for the discrepancy between the results might be a difference in the composi tion of the clinical materials

Our data indicate that there is some age de pendency in the response of omental tissue to nor adrenaline with the magnitude of response in versely correlated with increasing age Other au thors have attempted to correlate noradrenaline response in human adipose tissue with obesity (13 18 21) Such studies will require large num bers of subjects to distinguish the influence of obesity per se from the influence of unrelated factors such as age and sex

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GLUCOSE TOLERANCE AND PLASMA INSULIN IN MAN DURING ACUTE AND CHRONIC ADMINISTRATION OF NICOTINIC ACID

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tract Since nicotinic acid is known to interfere with the glucose metabolism a detailed study was made to lish whether insulin secretion is involved in this ac On chronic administration of nicotinic acid to hyper idemic subjects the fasting blood glucose and plasma immunoreactive insulin (IRI) concentrations rose significantly The elimination rate constant (K) of intravenous glucose decreased from an average of 1 88 to 1 07 The plasma IRI response increased but only in relation to blood glucose level Biguanide treatment did not improve the glucose utilization during nicotinic acid treatment.

A short term effect of the drug given as intravenous infusion to control subjects was a decrease of K value of iv glucose a slight increase of basal plasma IRI level and an unchanged plasma IRI response to glucose load In subjects maintained on total calory restriction or on high fat low-carbohydrate diet nicotinic acid did not cause any further decrease of the primarily low K value A similar lack of effect was present in patients with liver cirrhosis Also in these instances the IRI response was not modified by nicotinic acid

It is suggested that nicotinic acid impairs the hepatic glucose assimilation by increasing both glycogenolysis and gluconeogenesis through some action which is not related to antilipolysis Insulin secretion is slightly stimulated by the drug

Many reports (1 23 25) have indicated that administration of nicotinic acid to normal or even diabetic human subjects causes an improvement of carbohydrate metabolism In sharp contrast to this however the large doses of nicotinic acid used for the treatment of hypercholesterolemia have been rather convincingly demonstrated to result in an impaired glucose tolerance in non diabetic subjects (16) and to ameliorate diabetes mellitus (3 4 26) The reduction of glucose tolerance by nicotinic acid is a rather unexpected phenomenon in view of the fact that the drug inhibits lipolysis in adipose tissue and decreases the plasma FFA concentration (8) which should improve glucose tolerance by increasing the glucose assimilation in muscle (37) Also in adipose tissue nicotinic acid at least in vitro increases the uptake of glucose (24) In fact nicotinic acid has been shown to accelerate the disappearance of tracer glucose in rats (38) and to abolish the impaired removal of glucose caused by noradrenaline infusion in man (28)

The mechanism by which nicotinic acid influences the glucose metabolism has not been solved Lammers et al (22) have suggested that the impairment of the glucose tolerance might be due to an inhibition of the insulin secretion by the drug Jenkins (21) thought that because ketone bodies stimulate insulin secretion (25) the reduced ketone body production during nicotinic acid leads to a diminished output of insulin and this accounts for the impaired glucose tolerance To evaluate the role of insulin in the nicotinic acid induced changes of carbohydrate metabolism the effect of the drug was studied on the glucose tolerance and on the glucose stimulated plasma insulin response in hyperlipidemic patients treated with large doses of nicotinic acid and in normal fat fed and starved subjects as well as in patients with liver cirrhosis The results show that the glucose tolerance is decreased by the drug but that this is not associated with any inhibition of insulin secretion

MATERIAL AND METHODS

The chronic effect of nicotinic acid on glucose tolerance was determined in 14 hypercholesterolemic patients hospitalized for the investigation and treatment of their lipid disorder Eleven of the subjects had a pure familial hypercholesterolemia (Type II) while the remaining three patients had in addition a slight hyperglyceridemia. The pa

tients were kept on an ordinary hospital diet that provided about 40 of calories from carbohydrates The control intravenous glu ose tolerance test (IGTT 50 ml of 50% glucose) was carried out after the patients had been on the hospital diet for at least one week. Twelve of the subjects had normal K values while in the remaining two the fractional rate of glucos removal was less than 1.2 Nicotinic acid treatment was started with 1 g (Nicangin® *Draco Sweden) on the first day* 2 g on the second day and continued with 1 g three times daily This treatment reduced serum cholesterol within two weeks by an average of 25 and triglycerides by 30 After the maximal dose had been used for 10 to 14 days IGTT was repeated 14 hours after the last oral nicotinic acid dose and after 12 hours fasting Plasma FFA concentration was measured at the beginning of IGTT and at 50 min after the glucose injection Plasma immunoreactive insulin (IRI) was deter mined at 0 2 10 20 and 50 min and the blood glucose at 10 minute intervals up to 50 min

Oral glucose to erance tests (1 g of glucose/kg of body weight) were carried out in five of thes patients before and during the nicotinic acid treatment

To show whether biguanides are able to improve glucose to erance during nicotinic acid treatment the drug (Di bein retard® Astra Sweden) was given (50 mg twice a day) to seven additional hyperlipidemic non-diabetic sub jects who had been on the nicotinic acid treatment for 10 days IGTT was carried out prior to and 5–7 days after the start of the biguanide therapy

The acute effect of nicotinic acid on glucose to erance as first studied in five metabolically normal in patients who all showed normal K values in the IGTT The test as carried out on consecutive days with and without prior nicotinic acid infu ion (or vice versa) so that again every patient served as his own control Nicotinic acid (Nicangin®) 200 mg for each subject was given a a s ow intravenous injection over a period of 6 min Blood samples for glucose insulin and FFA ana ysis were drawn as indicated above and IGTT was started 30 min after the beginning of the nicotinic acid injection

The same experiments were also carried out in patients given a hi h fat diet in starving obese subjects and in pa tients with advanc d cirrhosis of the liver Five non diabetic pa ients with 50 to 70 overweight were put on a total fa t for 7–10 days IGTT with and without nico-tinic acid was carried out before and at the end of thi period The weight reduction ranged from 4 to 6 kg and all of the subjects showed a marked ketosis at the time of the second IGTT Five other non-obese non-diabetic patients were put on an isocaloric diet that provid d 80% *of calories from fat* (a mixture of soya bean oil and butter) 5% from carbohydrates and 15 from proteins IGTT was performed before and after 7 to 10 days on this diet An additional five patients with liver cirrhosis (two with Wilson s disease and three with excessive alcohol consumption) were studied The diagno is was verified with percutaneous needle biopsy of the liver As judged from c inical condition and laboratory find ngs the liver function appeared to be compensated in all but one sub-ject whose serum bilirubin and ammonia were clearly elevated

Analytical procedures The radio-iodination of insulin was carried out by the method of Hunter and Greenwood (14) and plasma IRI was d termined according to Morgan and Lazarow (27) The orthotoluidine method by Hyvärinen and Nikkila (20) was used for the measurement of blood glucose Triglycerides were determined by the method of Carlson (6) cholesterol according to Pearson et al (33) and FFA as presented by Trout et al (40)

RESULTS

Chronic effect of nicotinic acid

The results a e pre ented in Table I and they show that during the nicotinic acid treatment both fasting blood glucose and plasma IRI concentra tion increased significantly The fasting FFA level was however not changed The rate of glucose disposal as indicated by the K value of IGTT decreased in 12 out of 14 subjects and increased slightly in two The K value which before the treatment exceeded 1 2 in 12 patients was during nicotinic acid treatment below that level in ten cases although none of them manifested clinical diabetes The average K value was reduced from 1 88 to 1 07 by nicotinic acid

That the decreased glucose utilization was not due to a deficient secretion of insulin is shown by the plasma IRI response which tended to be even higher during than before the nicotinic acid ad minis ration However when the insulin response is expressed in relation to the corresponding blood glucose level i e a the ratio Δinsulin/Δglucose (39) there appeared to be no difference between the on and off nicotinic acid periods The average ratio at 2 min was 0 48 before and 0 45 during the therapy The initial level of plasma FFA and its fall w re of the same order in the two glu ose tolerance tests indicating that the impaired glucose tolerance could not be accounted for by different availability of free fatty acids to tissues

Since biguanides improve glucose *utilization* by p ripheral tissues IGTT was carried out during chronic nicotinic acid treatment without and with additional biguanide therapy to show whether the drug is able to counteract the effect of nicotinic acid on glucose tolerance As presented in Table II biguanide did not however significantly change the disposal rate of glucose although the plasma insulin response tended to be de reased by the drug The ratio Δinsulin/Δglucose was now smaller

e I *Effect of nicotinic acid treatment on fasting blood glucose on fractional removal rate (K) of intra glucose and on plasma insulin and FFA response to intravenous glucose (Mean ± S E)*

Treatment	Fasting blood glucose	*K* value	Plasma IRI response to intravenous glucose					Plasma FFA response	
			0 min	2 min	10 min	20 min	50 min	0 min	50 min
ne	84±2 3	1 88±0 24	16±2 2	64±9 2	51±5 2	39±4 2	32±5 2	576±75	287±35
Nicotinic acid[a]	93±2 0[b]	1 07±0 11[b]	25±3 3[b]	85±9 9	64±6	57±4 4	41±5 3	565±72	268±21

tinic acid 3 g/day at least for 10 days. The last dose was given 14 hours before IGTT (14 subjects)
$p<0.05$

Table II *Effect of biguanide (phenformin) treatment on the nicotinic acid induced changes of glucose and insulin metabolism (Mean ± S E)*

Treatment	Fasting blood glucose	*K* value	Plasma IRI response to intravenous glucose				
			0 min	2 min	10 min	20 min	50 min
Nicotinic acid	99±5	0 77±0 11	24±4	70±17	57±16	49±10	48±9
Nicotinic acid + biguanide	94±3	0 74±0 09	16±5	44±5	33±6	28±4	26±5

suggesting that glucose utilization per unit insulin was improved

The oral glucose tolerance test was also impaired during chronic nicotinic acid treatment as revealed by Table III Although the plasma insulin response to oral glucose was markedly increased during the therapy the ratio Δinsulin/Δglucose remained unaffected indicating that the insulin secretion was not influenced by the drug

Acute effect of nicotinic acid in control subjects

Intravenous infusion of 200 mg of nicotinic acid to normal human subjects maintained on the ordinary hospital diet did not change the blood glucose level within 30 min but elevated the plasma IRI concentration (Table IV) and in agreement with earlier findings (8) decreased plasma FFA concentration (Table V) after an initial slight but significant rise IGTT carried out 30 min after the nicotinic acid injection revealed that despite reduced initial FFA concentration the glucose disappearance rate was significantly slower than without nicotinic acid load (Table VI) The plasma IRI response expressed as the difference between basal and maximum levels was identical in both experiments (Table VII) The ratio Δinsulin Δglucose calculated for 2 minute value (Table VIII) also showed that the actual insulin response was not influenced by nicotinic acid Thus impaired glucose tolerance was not due to a poor stimulation of insulin secretion

Table III *Effect of chronic nicotinic acid treatment on blood glucose (mg/100 ml) and plasma IRI (μU ml) during oral glucose tolerance test (Mean ± S E)*

	Difference from pretreatment values				
	0 min	30 min	60 min	90 min	120 min
Blood glucose	+15±	38±4	34 7	9±4	8±
Plasma IRI	+3±1	14±3	17±3	13±1	9±3

Table IV *Effect of nicotinic acid infusion (200 mg) on fasting plasma IRI*

The figures give the mean difference ± S E (μU ml) from preinfusion value

Group ( )	Time min		
	10	20	30
Controls (5)	9 0±5 0	−7 0±3 4	8 0±1 9
Fasted (5)	−5 0± 5	−5 4± 9[a]	7 ±1 9
Fat fed (5)	5 6±6 8	0 8 1 8	−4 5±1 5
Cirrhotics (5)	−4 ±1 4	−6 0±3 7[a]	−5 4± 7[a]

Difference from control groups significant at p 0 05
= number of subjects

Table V *Effect of nicotinic acid and subsequent IGTT on plasma FFA (Mean±s E)*

Group	FFA μEq/l 0 min[a]	30 min[b]	80 min
Controls	335± 25	251± 24	228± 45
Fasting	1181±168[c]	816± 98[c]	1409±215[c]
Fat fed	1019±142[c]	569±102[c]	779±256[c]
Cirrhotic	523±115	261± 48	340±100

[a] Before nicotinic acid infusion
[b] Before glucose infusion
[c] Significant difference ($p<0.05$) from corresponding control values

Acute effect of nicotinic acid in starved fat fed and cirrhotic subjects

To learn whether the diabetogenic effect of nicotinic acid could be demonstrated also when the basal utilization of glucose was depressed the experiments were repeated in starved and fat fed patients Particularly to show the role of the liver experiments were also performed in cirrhotic patients whose liver is known to form and utilize glucose less than normal

The fasting blood glucose level was not changed any of these groups during 30 min after the of nicotinic acid Basal IRI level was ghtly decreased by nicotinic acid the change at 30 min being in all three groups significantly different from that in controls (Table IV) Thus in contrast to normal subjects the basal utilization of glucose in relation to plasma insulin level appears to be improved by nicotinic acid in starved fat fed and cirrhotic subjects The FFA level, which in starved and fat fed subjects was originally higher than in the other groups decreased significantly during nicotinic acid therapy in every instance but remained still higher than normal in fasted and fat fed patients (Table V)

Table VI shows that in contrast to normally fed control subjects the glucose disposal rate was uninfluenced by the drug in all these three groups The average change of the K values differed significantly in starved and cirrhotic subjects from those in controls suggesting that at least in these two conditions the factor was absent which normally is responsible for the acute impairment of glucose utilization by nicotinic acid load Table VI demonstrates further that after nicotinic acid the K values were of the same order of magnitude in controls and in starved and fat fed patients despite 2–5 fold higher initial and final plasma FFA concentrations in the two latter groups (Table IV)

The response of plasma IRI in the IGTT was low after total fasting and high fat diet the increment being at 2 min but not later significantly less than in normally fed controls (Table VII) The IRI response was not modified by nicotinic acid In spite of still existing hyperinsulinemia the FFA level tended to increase at 50 min after the IGTT in subjects with superimposed nicotinic acid load (Table V) while low FFA levels were observed in patients without prior nicotinic infusion or with chronic nicotinic acid treatment (Table I) Although the ratio Δinsulin/Δglucose (which was especially low in starved subjects) was not significantly modified by nicotinic acid the values at 2

Table VI *Effect of nicotinic acid infusion on K values of IGTT*

Case no	Controls Before	Controls After	Starved Before	Starved After	Fat fed Before	Fat fed After	Cirrhotic Before	Cirrhotic After
1	1 23	0 99	1 08	1 19	0 87	0 64	1 54	1 65
2	1 39	1 33	0 87	0 87	3 30	1 47	1 34	1 61
3	2 16	1 42	0 98	0 88	0 66	0 95	1 78	1 45
4	1 28	0 82	0 67	0 84	0 95	0 90	1 10	1 14
5	1 87	1 21	0 93	1.26	1 19	0 96	1 19	1 14
Mean±s E	1.59 ±0 17	1 15 ±0 11	0 90 ±0 08	1 02 ±0 08	1 39 ±0 48	0 98 ±0 13	1 39 ±0 13	1 41 0 08
Difference ±s E	−0 43±0 13		+0 13±0 09[a]		−0 41±0 36		+0 0 ±0 09[a]	

[a] Difference from control group significant at $p<0.05$

ıble VII *Increment of plasma insulin (ΔIRI μU/ml) during intravenous glucose tolerance test with and with- superimposed nicotinic acid administration (Mean ± s e)*

oup	Nicotinic acid	2 min	10 min	20 min	50 min
ntrols	+	69 0±13 7	29 8± 6 6	18 6± 7 7	12 2± 2 8
	−	70 0±14 8	33 2±10 5	21 8± 7 2	18 2± 2 4
ı	+	21 8± 4 9[a]	21 6± 6 9	22 2± 6 5	9 0± 3 2
	−	17 6± 3 7[a]	11 4± 4 0	9 6± 2 0	.0 2± 5 6
t fed	+	30.2± 9 0[a]	21 6± 6 4	13 6± 4 9	11 0± 3 6
	−	26 0± 7 0[a]	13 6± 5 8	5 4± 3 5	14 6± 6 2
rrhotic	+	38 0±17 3	25 2± 6 7	18 6± 8.2	25 4± 7 2
	−	51 0±23 3	49 8±32 2	79 4±57 5	30 4±11 5

ıfference from controls significant at $p < 0\ 05$

n (Table VIII) were only about one fourth of in controls The finding combined with the cal postnicotinate K values in control fasted fat fed subjects suggests that utilization of glucose per unit plasma insulin is arkedly better after nicotinic acid in fasted and ed cases with high plasma FFA concentration in normal subjects with low FFA level

DISCUSSION

The basic biochemical mechanism of the different c actions of nicotinic acid is unknown In pose tissue it induces a very effective antilipoly is (5 12) and increases the assimilation of glucose 19 24) but it has not been shown whether these two effects are causally interrelated or have a com biochemical basis Changes noted in other issues and in blood may be secondary to these ons on fat cell but some of them may equally well be attributed to other primary pharmacologi influences of the drug Among the latter is the prompt decrease of liver and muscle glycogen (2 38) reduction of blood and liver lipids (7) and variable response of blood glucose and glucose

Table VIII *Effect of nicotinic acid infusion on the ratio Δinsulin/Δglucose at 2 min after intravenous ucose injection (Mean ± s e.)*

Gro p	Before NA	After NA
Controls	446 ± 086	452 ± 087
Starved	167 ± 061	102 ± 043
Fat fed	245 ± 084	118 ± 034
Cirrhotics	231 ± 094	?90 ± 136

Difference from control group signif cant at $p < 0\ 05$

tolerance On account of the reduced plasma FFA level one should expect that the uptake of glucose in skeletal muscle is increased by nicotinic acid (37) but direct evidence of this has never been offered Such an assumption is supported how ever by the finding that nicotinic acid enhances the RQ in both resting and exercising man (11 18) and in dog (32) The finding that nicotinamide has a similar effect as nicotinate on blood glucose and liver glycogen (29) but has no effect either on lipolysis (5 34) or on blood lipids actually sug gests that nicotinic acid itself might have several primary metabolic actions

The immediate effect of nicotinic acid on the fasting blood glucose concentration and on the elimination rate of a glucose load has been the subject of some controversy which however may be partly ascribed to different experimental con ditions Significant decrease of blood glucose level on administration of nicotinic acid have been re corded in *fasted* normal and diabetic rats (7 9 15 38) in *fasting* diabetic dogs (32) in normally fed mice (2) and in diabetic human subjects (10) That insulin might function as one mediator in these effects of nicotinic acid is shown by the slightly but significantly increased plasma immuno-reactive insulin level found here for human sub-jects and previously in rats (30) On the other hand no constant change of fasting blood glucose level of normal men was noted in the present study nor in an investigation of diabetics (17) nor by Paul et al (32) in normal fasting dogs Also increases of blood glucose level on nicotinic acid infusion have been reported in normally fed and starving men (36)

Nicotinic acid in vivo accelerates the removal

and oxidation of glucose (32 38) which finding fits well to the effects observed in isolated adipose tissue or fat cells (19 24) and probably accounts for the fall of blood glucose In non diabetic subjects the increased utilization is compensated by an enhanced hepatic output of glucose which in fed state could come partly from stimulated breakdown of glycogen (24 38) partly from accelerated gluconeogenesis and in fasting or high fat feeding from the latter source only The alterations of blood glucose level represent a net effect of all these three metabolic actions of the drug and this makes it understandable that rather variable changes of blood glucose have been reported It is somewhat unclear whether nicotinic acid is able to enhance the peripheral uptake of glucose also in the absence of insulin Root and Ashmore (38) observed an accelerated disappearance of labeled glucose after nicotinic acid in alloxan diabetic rats while Paul et al (32) could not demonstrate this in pancreatectomized dogs Glucose entry rate was not stimulated in the latter instance either but was on the contrary decreased The antilipolytic activity of nicotinic acid is not dependent on insulin (31)

In view of the influence of nicotinic acid on the basal turnover rate of blood glucose it seems difficult to understand why the disposal of exogenous glucose loads behaves just in an opposite way i e the elimination is retarded The results of plasma insulin and FFA assays presented above indicate that neither a deficient insulin release nor a high FFA concentration can afford an explanation

There is also no reason to assume that the assimilation of glucose by adipose tissue should be inhibited by some other mechanism causing insulin resistance This is best shown by the observations in vitro mentioned above (19 24) and by the finding that in patients on chronic nicotinic acid treatment the plasma FFA response to intravenous glucose is normal in spite of a clearly retarded glucose removal There thus remain the possibilities that glucose uptake by muscle or liver or both is inhibited A defective glucose utilization in skeletal muscle after nicotinic acid could be ascribed to inhibition of muscle hexokinase by the increased production of glucose 6-phosphate from stimulated glycogenolysis but the rise of RQ in exercising man during nicotinic acid infusion (11) definitely tells against this possibility The only plausible explanation for the decrease of glucose tolerance is an impaired hepatic assimilation This view is well supported by the absence of effect of nicotinic acid in cases with liver cirrhosis

The factors other than blood glucose level and glucokinase activity determining the hepatic glucose uptake are not well defined It seems however that stimulated gluconeogenesis and glycogen breakdown are accompanied by a decrease of glucose uptake by the liver even in the presence of hyperglycemia and active glucokinase The primary point of action of nicotinic acid in both these processes has not been explored but the lack of its effect on glucose tolerance in conditions when glycogen stores have been exhausted and gluconeogenesis is primarily high—as in fasting and fat fed subjects of the present series and in diabetics studied by Carlson and Östman (10)—suggests that some rapid regulatory device switches the hepatic metabolism from glucose uptake to glucose production On chronic feeding of nicotinic acid this same factor probably causes enzyme induction which leads to a constant hepatic over production of glucose hyperglycemia and impaired glucose elimination which are no longer dependent on the actual presence of nicotinic acid in the circulation That the enhanced glucose production did not appear to be related to actual availability of free fatty acid and that the same effect is obtained with non lipolytic nicotinamide suggests that the primary gluconeogenetic factor might be the changed nucleotide status in the liver Nicotinic acid as well as nicotinamide results in a marked increase in hepatic NAD and NAPD levels (13)

Note added to proof Some of the results of the present study have been presented at the Meeting of the Scandinavian Society for the Study of Diabetes (Stockholm 1968 abstracted in Diabetologia 4 311 1968) Lurcki observed more recently (1969 personal communication) no effect of nicotinic acid on plasma IRI in anesthetized dogs but recorded a frank increase of the plasma glucagon level

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BLOOD VOLUME AND EXCHANGEABLE SODIUM DURING TREATMENT OF HYPERTENSION WITH GUANETHIDINE AND HYDROCHLOROTHIAZIDE

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Abstract Antipressor treatment with guanethidine for 8 to 14 days produce a significant average increase in blood volume and exchangeable sodium of 382 ml (8 5) and 210 mEq (7 %) respectively

Subsequent supplementary treatment with hydrochlorothiazide resulted in a normalization of the above mentioned parameters both during short term and long term management

It is proposed that these effects are of importance for the continued hypotensive effect of the thiazides

It has been known for several years that a number of antipressor agents can cause fluid retention This applies to ganglionic blocking agents (7 22 23) *rauwolfia* alkaloids (16) and α methyldopa (10 11 15) Fluid retention is especially pronounced during treatment with guanethidine (4 19 20 25) and a significant increase in blood volume (24 26) and exchangeable sodium (26) results The effects of thiazide preparations have been demonstrated in several studies which all show that exchangeable sodium blood volume and extra cellular space are reduced after treatment for less than 2 to 3 weeks (2 3 5 9 12 27) The majority of these investigations show however that these parameters tend to become normal during long term treatment According to Wilson and Freis (29) plasma volume and extracellular fluid reach pretreatment values *in* the course of 6–12 months of treatment Other investigators find that plasma volume reaches the pretreatment level after 1 to 2 months of treatment (2 9) Lauwers and Conway (18) could not demonstrate a reduction of extracellular fluid and exchangeable sodium after 26 to 60 days of treatment In spite of this re hydration during long term treatment Wilson and Freis (29) found that on discontinuance of the drug an additional increase in plasma volume extracellular fluid and body weight occurred during the course of a week This discontinuance took place on the average after 5 4 months of treatment

One of us (11) on the other hand found a significant reduction in plasma volume and total blood volume after both 1 to 2 weeks and 3 months of treatment with hydrochlorothiazide in ten hyper tensive patients without there being any sign of a decreasing effect on these parameters Exchange able sodium was also found to be significantly reduced after both short and long term treatment but here the effect seemed to be diminishing

The objective of the present study has been to follow variations in total blood volume extra cellular space and exchangeable sodium during treatment with guanethidine alone and particularly during short term and long term combined therapy with guanethidine and hydrochlorothiazide as such a study has not previously been undertaken

MATERIAL AND METHODS

Total blood volume was calculated as the sum of the erythrocyte and the plasma volume using a double isotope technique with ^{51}Cr and ^{131}I albumin as previously de scribed (13) Extracellular space and exchangeable sodium were determined with ^{24}Na which was given intravenously (approx. 100 μC) after a standard had been taken Activity in the serum and urine was measured after 3 and 24 h

Extracellular space in liters was calculated from the 3 hour values, as these values are usable as an index of extracellular space (8) The following formula was used,

$$\frac{\text{administered activity} - \text{activity in urine}}{\text{activity in plasma per ml} \times 1000}$$

Table I *Exchangeable sodium serum sodium extracellular space total blood volume blood pressure and weight in 20 hypertensive patients during treatment with guanethidine alone and in combination with hydrochlorothiazide*

Pat no	Sex	Age (y)		Before treatment	After 8-14 days treatment with guanethidine	After 8 14 days combined treatment	After 3 15 months combined treatment
1	♂	39					(4 mo)
			TES	2861	3090	3108	3095
			TES/kg	37 7	39 2	38 9	37 5
			PS	141 0	142 0	141 0	140 5
			ECF	18 85	19 87	19 79	19 5
			BP	173	130	1?3	1.5
			W	75 8	78 9	79 9	82.5
2	♂	40					(5 mo)
			TES	3676	3571	3385	3263
			TES/kg	47 7	45 4	44 5	38 8
			PS	145 5	141 5	140 0	140 0
			ECF	21 43	22 48	21 01	21 50
			BP	165	148	153	153
			W	76	77 5	76 1	84 0
3	♀	70					
			TES	2258	2617	2403	—
			TES/kg	31 5	35 6	33 4	—
			PS	140 0	146 0	14? 25	—
			ECF	14 30	16 49	14 99	—
			BP	170	150	140	—
			W	71 7	73 5	71 9	—
4	♂	65					(3 mo)
			TES	3101	3168	3084	2818
			TES/kg	52 3	51 3	52 3	49 4
			PS	145 0	145 0	144 5	147 0
			ECF	18 04	20 32	19 13	—
			TBV	4887	—	5067	4657
			BP	160	140	125	153
			W	59 3	61 7	59 0	57 0
5		45					
			TES	2185	2?86	2132	—
			TES/kg	41 6	4? 3	39 1	—
			PS	144 25	145 0	138 0	—
			TBV	3055	3371	3162	—
			BP	135	123	113	—
			W	5? 5	54 1	54 5	—
6	♀	58					
			TES	3016	3065	2583	—
			TES kg	39 3	39 9	34 9	—
			PS	147 25	145 0	139 0	—
			TBV	3933	4444	3711	—
			BP	170	138	145	—
			W	76 7	76 8	74 1	—
7	♂	54					(4 mo)
			TES	3 10	3362	3169	—
			TES kg	42 9	44 2	42 5	—
			PS	145 0	148 75	151 25	—
			TBV	5?35	5197	5194	5168
			BP	153	138	120	138
			W	74 8	76 0	74 6	74 8
8	♂	60					(3 mo)
			TES	2959	3140	2844	848
			TES kg	47 0	48 7	46 1	44 5
			PS	143 0	145 0	143 75	145.5
			ECF	18 56	19 99	17 96	17 3
			TBV	4472	—	—	4349
			BP	193	143	135	175
			W	63 0	64 5	61 7	64 0

Table I *Continued*

Pat no	Sex	Age (y)		Before treatment	After 8–14 days treatment with guanethidine	After 8–14 days combined treatment	After 3–15 months combined treatment
9	♀	54					(5 mo)
			TES	2249	2420	2146	2230
			TES/kg	35 0	36 9	33 6	33 6
			PS	145 25	149 0	142 5	143 5
			ECF	13 53	15 01	13 73	14 23
			TBV	3439	3779	—	—
			BP	153	138	128	118
			W	64 2	65 5	64 2	66 3
10	♂	50					(9 mo)
			TES	2963	3093	2681	2766
			TES/kg	41 1	44 5	39 7	39 0
			PS	143 75	142 0	140 25	14 0
			ECF	18 23	19 54	16 98	17 45
			TBV	4651	5012	—	4413
			BP	185	170	150	155
			W	67 2	69 5	67 5	71 0
11	[illegible]	47					(6 mo)
			TES	2887	3253	—	3078
			TES/kg	31 5	35 9	—	34 4
			PS	145 25	145 75	—	146 0
			ECF	17 92	20 44	—	18 74
			TBV	4662	4923	—	4921
			BP	130	135	—	110
			W	91 5	90 6	—	87 0
12	♂	58					(5 mo)
			TES	2884	3245	2864	2938
			TES/kg	42 3	46 0	41 3	39 7
			PS	138 5	140 0	141 0	140 0
			ECF	18 7	—	18 57	19 33
			TBV	4601	5273	4788	5146
			BP	170	148	143	155
			W	68 2	70 6	69 4	74 5
13	o	63					(4 mo)
			TES	3502	4318	3315	3214
			TES/kg	46 8	55 0	44 7	40 4
			PS	145 0	141 25	140 25	140 0
			ECF	21 14	25 22	21 27	20 65
			TBV	5024	5844	5012	5128
			BP	163	145	140	143
			W	74 8	78 5	74 1	79 5
14	♂	59					(3 mo)
			TES	3083	3150	3011	930
			TES/kg	39 9	40 5	39 1	38 0
			PS	143 75	146 0	140 5	140 0
			ECF	18 81	19 78	18 71	—
			TBV	4515	4529	4291	4336
			BP	145	123	113	128
			W	77 2	77 7	77 0	77 1
15	♂	48					(11 mo)
			TBV	4986	5212	—	4777
			BP	138	118	—	130
			W	60 0	61 5	—	61 0
16	o	52					
			TBV	4824	5 05	4515	—
			BP	143	140	140	—
			W	66 4	66 8	66 8	—

1⁼ – 83 998

Table I *Continued*

Pat no	Sex	Age (y)		Before treatment	After 8-14 days treatment with guanethidine	After 8 14 days combined treatment	After 3 35 months combined treatment
17	o	44					(7 m)
			TBV	5641	6197	—	5341
			BP	148	173	—	1 8
			W	80 8	81 8	—	80 5
18	o	61					(15 mo)
			TBV	5885	6365	—	5947
			BP	153	143	—	145
			W	73 5	74 5	—	75 0
19		57					(13 mo)
			TBV	3786	4342	—	3556
			BP	158	140	—	130
			W	54 5	55 3	—	56 4
20		45					(13 mo)
			TBV	3517	3795	—	3675
			BP	130	115	—	105
			W	50 5	60 0	—	55 7

Abbreviations

TES – total exchangeable sodium in mEq
TES kg total exchangeable sodium per kg body weight
PS plasma sodium in mEq liter
ECF – extracellular fluid in liters
TBV total blood volume in ml
BP – blood pressure $\left(\frac{\text{systolic} \quad \text{diastolic}}{2}\right.$ in supine position (mm Hg)$\left.\right)$
W – body weight in kg

nd in the same way exchangeable sodium was calculated rom the 4 hour values.

$$\frac{\text{administered activity} \quad \text{activity in urine}}{\text{activity in plasma per ml} \quad 1000}$$

serum sodium in mEq l

S rum sod um wa determined in quadruplicate by flame photome ry

In cases in whi h blood volume extracellular fluid and exchangeable sodium were determined the sodium deter minati ns were done first and the blood volume measured immediately afterward [?]I activity was counted 1 to ? days after the bl od samples had been taken while count ing of the [?]Cr activity was postponed for about a week to ensure that es ntially all the Na activity had de cayed. All counts were done in a well t pe scintillation detec or and the counting of standards, bl od serum samples and background values was continued until the standard deviation for the total cal ulation was less than 2

Twenty patients with severe hypertension 1 men and eight women, were studied Two had papilledema three fresh exudates, and three had hemorrhages, while the others only had vascular eyeground changes. None had heart failure before treatment. Renal function was es i mated by means of creatinine clearance 11 had greater than 70 ml min, three between 50 and 70 ml min and in five creatinine clearance was between 30 and 50 ml min The twentieth patient had a serum creatinine of 1 0 mg 100 ml The patients were first treated with guanethi ne alone in a dose of 1 5 to 100 mg per day for 8 to 14 days after whi h combination therapy w th h drochloro thiazide 25 to 75 mg daily was started The add n of hydrochl rothiazide made it necessary in many cases to reduce the amount of guanethidine gi en th final dose of the latter drug being thereby 10 t 0 mg a day Stu lies were carried out bef re and after 8 to 14 da s of treatment with guanethidine and were repea ed af er 8 t 14 day of comb nation therapy and aga n fter t 1 n the a erage 7 months of combined treatment. It was not po sib e to arry ut all of the planned studies in all of the patient In some cases only b ood v me in others nly odium measurements were o ne

RESULTS

All of the relevant results are summarized in Table I

Blood volume

Average blood volume and blood pressure in 15 patients before and after short term treatment with

Table II *Mean blood volume (MBV) and the average mean blood pressure (MBP)*[a] *in 15 hypertensive subjects before and after 8–14 days of treatment with guanethidine*

	Before treatment	During treatment	Change (per cent)	S E	P
MBV (ml)	4517	4899	+382 (+8 5)	±68	<0 001
MBP (mm Hg) (supine)	152	135	− 17 (−11)	± 2	<0 01

[a] Mean blood pressure = $\frac{\text{systolic} + \text{diastolic}}{2}$

guanethidine are shown in Table II Treatment resulted in a highly significant average increase in blood volume of 382 ml accompanied by a significant fall in mean blood pressure of 17 mm Hg Variations in blood volume in individual patients are shown in Fig 1 In only two cases was the blood volume essentially unchanged in the others it increased

In seven patients the same parameters were compared after guanethidine treatment alone and after 8 to 14 days of combined guanethidine hydrochlorothiazide treatment As shown in Table III there was a significant reduction in blood volume (454 ml) and a non significant fall in mean

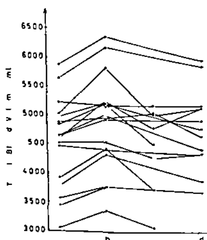


Fig 1 Total blood volume in 17 hypertensive subjects. (*a*) Untreated (*b*) During treatment with guanethidine (*c*) After 8–14 days of combined treatment with guanethidine and hydrochlorothiazide (*d*) After 3–15 months of combined treatment.

Table III *Mean blood volume and the average mean blood pressure in seven hypertensive subjects after 8–14 days treatment with guanethidine alone and after 8–14 days combined treatment with guanethidine and hydrochlorothiazide*

	Guanethidine alone	Combined treatment	Change (per cent)	S E	P
MBV (ml)	4838	4384	−454 (−9 4)	±106	<0 01
MBP (mm Hg) (supine)	136	130	− 6 (−4)	± 3	>0 05

blood pressure (6 mm Hg) The reason for this is that it was necessary in several cases to reduce the dose of guanethidine in order to avoid too great a fall in blood pressure as earlier mentioned Fig 1 illustrates variations in blood volume in individual patients There was a marked reduction in six cases while blood volume remained unchanged in the rest

Table IV (11 patients) shows that blood volume remains significantly reduced after long term treatment (3–15 months) with guanethidine and hydrochlorothiazide in relation to the values found during guanethidine treatment alone (316 ml) while there is no change in the level of blood pressure reduction In four cases the fall in blood volume was less than 100 ml in the others it was more marked (Fig 1)

Our studies have also shown that blood volume remains unchanged after both long term and short term combined guanethidine hydrochlorothiazide therapy when the values obtained before treatment were compared to those obtained after

Table IV *Mean blood volume and the average mean blood pressure in 11 hypertensive subjects after 8–14 days treatment with guanethidine alone and after 3–15 months combined treatment with guanethidine and hydrochlorothiazide*

	Guanethidine alone	Combined treatment	Change (per cent)	S E	P
MBV (ml)	5153	4837	−316 (−6 1)	±7	0 005
MBP (mm Hg) (supine)	136	134	− 2	± 3	0 5

Table V *Mean exchangeable sodium (MES) mean plasma sodium (MPS) and mean weight (MW) in 14 hypertensive subjects before and during short term treatment (8–14 days) with guanethidine*

	Before treat ment	During treat ment	Change	S E	P
MES (mEq)	2913	31_3	+210	±59	<0 005
MPS (mEq l)	143 75	144 45	+0 70	±0 77	>0 30
MW (kg)	70 9	7? 5	+1 60	±0 32	<0 001

Extracellular fluid and exchangeable sodium

Exchangeable sodium increased significantly (mean increase 210 mEq or 7% $p < 0.005$) in 14 patients during treatment with guanethidine alone and there was an average weight increase of 1 60 kg (Table V) As shown in Fig 2 there was an increase in exchangeable sodium in all patients except one The 3 hour ²⁴Na space was determined in ten patients and a highly significant increase of 1 83 l ($p < 0.001$) was found together with a mean weight increase of 1 70 kg The increase in extracellular space varied from about 1 to almost 4 l (Fig 3)

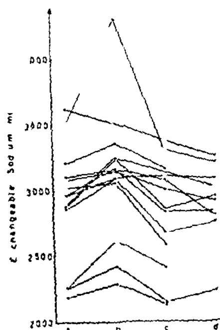


Fig 2 Exchangeable sodium in 14 hypertensive subjects. (a) Untreated (b) During treatment with guanethidine (c) After 8–14 days of combined treatment with guanethidin and hydrochlorothiazide (d) After 3–9 months of combined treatment.

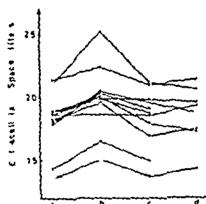


Fig 3 Extracellular space in 11 hypertensive subjects (a) Untreated (b) During treatment with guanethidine (c) After 8–14 days combined treatment with guanethidine and hydrochlorothiazide (d) After 3 9 months combined treatment

In 13 cases conditions during guanethidine treatment alone were compared to those during short term guanethidine hydrochlorothiazide combination therapy (Table VI) The reduction in exchangeable sodium was significant (288 mEq $p < 0.005$) Fig 2 shows that exchangeable sodium was reduced in 12 patients and that in only one was there a small increase In the nine cases in which the 3 hour ²⁴Na space was determined a mean reduction of 1 69 l ($p < 0.005$) was found with a mean weight decrease of 1 7 kg In the individual patients there was a slight increase in extracellular space in one and a reduction of 1 to 4 l in the other eight (Fig 3)

In ten cases exchangeable sodium after guanethidine treatment alone was compared to values after 3 to 9 months of combination therapy with hydrochlorothiazide Table VII shows that there was a significant reduction of 331 mEq ($p < 0.01$)

Table VI *Mean exchangeable sodium mean plasma sodium and mean weight in 13 hypertensive subjects after 8 14 days treatment with guanethidine alone and after 8 14 days combined treatment with guanethidine and hydrochlorothiazide*

	Guanethidine alone	Combined treatment	Change	S E	P
MES (mEq)	3113	?8 5	-288	75	<0 005
MPS (mEq l)	144 39	141 87	2.5	0 83	0 0[illegible]
MW (kg)	71 1	69 6	1 5	0 36	<0 005

Table VII *Mean exchangeable sodium mean plasma sodium and mean weight in ten hypertensive subjects after 8–14 days treatment with guanethidine alone and after 3–9 months combined treatment with guanethidine and hydrochlorothiazide*

	Guanethidine alone	Combined treatment	Change	S E	*P*
MES (mEq)	3240	2909	−331	±92	<0 01
MPS (mEq/l)	143 80	142 68	− 1 12	±0 82	>0 20
MW (kg)	73 5	74 3	− 0 8	±1 07	>0 40

and a non-significant weight increase of 0 8 kg Variations between individual patients are shown in Fig 2 Three hour ²⁴Na space was determined in seven patients and a significant mean reduction of 1 87 1 ($p<0\ 02$) was found Sodium space remained unchanged in one patient but decreased in the other six (Fig 3)

These studies have also shown that the values for exchangeable sodium are the same before and after long term as well as short term combined therapy with guanethidine and hydrochlorothiazide There is however a tendency to weight increase during long term combined therapy and since there is no change in extracellular space this is possibly a reflection of a true increase in total body mass

Changes in serum sodium were on the average quite modest Only when serum sodium values during guanethidine treatment alone are compared to those during short term combination treatment with hydrochlorothiazide is a significant reduction found (2 52 mEq/l ± 0 83 mEq/l $p<0\ 02$ (Table VI)

DISCUSSION

This study showed that there is an increase in blood volume extracellular space and exchangeable sodium during guanethidine treatment In contrast to Smith (26) we found that changes in blood volume and exchangeable sodium tended to be parallel That this investigator did not find the same relationship may perhaps in part be due to the fact that he calculated blood volume on the basis of the plasma volume and the hematocrit value while in the present study the erythrocyte volume and the plasma volume were measured independently with a double isotope technique Determinations of the extracellular space perhaps indicated that weight increase is caused by an increase in extracellular fluid though this cannot be said with certainty without determinations of total body water

The mechanism behind this salt and water retention is still not clear Vlasteris and Dustan (28) and Lauler et al (17) believe that it is determined by an accentuation of the normal postural retention of sodium This leads to salt and water retention during the day which must be compensated for by an increased excretion of these substances at night If diurnal retention is greater than nocturnal excretion the net result is a retention of salt and water According to Birkenhager et al (1) this is particularly seen in patients with a marked endogenous diurnal excretory rhythm in whom nocturnal salt and water excretion may be insufficient Smith (26) proposed the theory that guanethidine and other hypotensive drugs may stimulate the active tubular reabsorption of sodium In this way salt and water retention would be independent of the effect these drugs have on glomerular filtration and renal blood flow

In view of the effects of thiazide treatment in hypertension it is no surprise that combination therapy with guanethidine and hydrochlorothiazide produced a significant reduction in blood volume extracellular space and exchangeable sodium Previous studies have shown that there is a similar effect on blood volume when a thiazide is added to the therapeutic regimen of a patient being treated with a ganglionic blocking agent (23) Clinical experience with guanethidine and thiazides has shown corresponding effects in the form of weight loss and an increased excretion of sodium chloride and water (20) The agreement between weight loss and reduction of the extracellular space demonstrated in the present study seems to indicate that the most important homeostatic changes take place in that compartment

We have found that hydrochlorothiazide induced reduction of blood volume and exchangeable sodium remains essentially unchanged even with long term (3–15 months) combination therapy The explanation for this may be that guanethidine gradually loses its salt and water retaining effect On the other hand Smith (26) has shown that the increase in blood volume and exchangeable sodium produced by guanethidine does not change greatly

during the first year of treatment In addition clinical experience has shown that marked salt and water retention can appear at any time during treatment (20 25)

The mechanism by which thiazides produce their hypotensive and potentiating effects has not yet been explained There is general agreement that the initial reduction of blood volume and possibly of extracellular space is the most important factor responsible for their effect with short term treatment (3 5 29) As mentioned above most investigators find that the effects on blood volume extracellular fluid and exchangeable sodium disappear in the course of weeks to months (2 9 17 29) Thus other theories have been proposed to explain the long term hypotensive action of the thiazides e g that these drugs with long term treatment reduce peripheral resistance and thereby blood pressure by decreasing the salt and water content of the arteriolar walls (18) It is however likely that other factors also play a role It has been shown for example experimentally as well as in man that chlorothiazide reduces the vascular response to noradrenalin (6 14)

The present investigation suggests that a reduction in blood volume extracellular space and exchangeable sodium is also important in long term thiazide therapy in reducing blood pressure This is in agreement with our previous findings (12) In addition this reduction could be demonstrated both in patients treated with thiazides alone and those treated with thiazide and guanethidine

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THE POSTURAL PLASMA RENIN ACTIVITY RESPONSE IN ESSENTIAL HYPERTENSION AND PRIMARY HYPERALDOSTERONISM

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Abstract Supine and standing values of plasma renin activity (PRA) and colloid osmotic pressure (COP) were measured in 20 essential hypertensive patients including five malignant cases and in five patients with signs of primary hyperaldosteronism

The groups were compared mutually and to 22 normal controls of corresponding age and sex

Mean supine plasma renin activity (PRA) in essential benign hypertensives was significantly higher than normal Mean supine PRA in malignant essential hypertensives was significantly higher than in benign essential hypertensives Mean supine PRA in primary hyperaldosteronism was significantly lower than in benign essential hypertensives. In hypertensives with grade I–III a significant linear correlation between Δplasma colloid osmotic pressure and ΔPRA appeared The slope of the line was significantly less than the line for normal controls signifying a lesser postural response of PRA in this group In this respect there was no distinction between essential hypertensives and patients with primary hyperaldosteronism The decreased postural PRA response is considered to be a sequel to the hypertensive state

It has been established that plasma renin activity (PRA) in primary hyperaldosteronism is low or undetectable and the reaction to stimuli (e g changes in posture and/or salt depletion) is subnormal or completely abolished (3 4 5 6)

It has been claimed that hypokalemia is no constant finding in primary hyperaldosteronism (6)

Provided that a suppressed response in PRA to changes in posture or salt depletion is adequately specific for primary hyperaldosteronism PRA measurements during the above mentioned stimuli would be suitable as a screening test in the hypertensive population

The answers given to this important question up to the present show the occurrence of a number of false positives among patients with essential hypertension (7 16 23)

We have demonstrated a pronounced correlation between ΔPRA and hemoconcentration from supine to erect posture in normal individuals (20)

It was therefore thought of interest to study this correlation in essential hypertensives and in patients with primary hyperaldosteronism aiming at a more quantitative description of depression of the postural PRA reaction

MATERIAL

Normal controls

Twenty two individuals aged 19–67 five females and 17 males The individuals were healthy hospital employees and patients hospitalized for minor conditions unrelated to the cardio-vascular or renal system

Essential benign hypertensives

Sixteen individuals aged 22–63 two females and 14 males These patients showed no signs of accelerated course of hypertension Symptomatic hypertension" was excluded by the following examinations microscopy of urine se c eatinine i v pyelography renal arteriography (if indicated) urinary excreation of vanillin mandelic acid adrenaline and noradrenaline urinary excreation of ketosteroids and ketogenic steroids plasma electrolytes. There was no history of kidney disease or cardiac failure Examination of the fundi showed grade I–III (Keith Wagener) Antihypertensive treatment was discontinued at least 7 days prior to the examination

Essential malignant hypertensives

Four individuals, aged 20–45 three females and one male These patients had a severe progressive hypertension The retinal changes were grade IV As in the previous group any known cause of the hypertension was ruled out. All

Table 1 *Supine PRA (45 min recumbent) aldosterone secretion rate and plasma sodium and potassium after one week of normal salt intake (80 mEq Na⁺ 70 mEq K⁺ except case 25 investigated on liberal salt intake) and the same parameters after 4–6 days of salt loading (200 mEq Na 70 mEq K⁺ except case 47 who had 193 mEq K⁺) Three of the patients were investigated twice with an interval of 1–3 months*

Case no	Normal salt intake PRA$_0$	Aldo secr rate (μg/24 h)	Plasma Na⁺	Plasma K⁺	Salt load PRA$_0$	Aldo secr rate (μg/24 h)	Plasma Na⁺	Plasma K⁺
7								
1st invest	8	130	137	3 8	0	340	140	3 5
2nd invest	18	258	143	3 2				
17								
1st invest	10	453	146	2 8				
2nd invest	0	1464	147	3 2	0	308	144	3 0
25								
1st invest	4	208	143	3 7				
2nd invest	0	125	142	3 3	0	140	142	3 5
43								
1st invest	4	140	144	3 8	0	358	145	2 9
47								
1st invest	4	1937	145	1 8	0	558	145	3 1

the patients were hypokalemic (2 6–3 5 mEq/l) Drug treatment was discontinued 7 days prior to the examina tion

atients with signs of primary hyperaldosteronism

,ive individuals aged 31–55 three females and two males In all five patients suspicion was aroused either by spon taneous hypokalemia (cases 17 43 and 47) or easily in duced hypokalemia (gastroenteritis case 7 and diuretic therapy case 5) These patients are included in this group because 1) supine PRA was low or undetectable) aldosterone secretion rate was increased at least in one of the determinations (> 150 μg 24 h) 3) on salt load the aldosterone secretion rate was either not normalized or not suppressed if in normal range before salt loading (Table 1) These criteria are according to Luets her (17) All patients had fundoscopic changes grade I–II normal kidney function there were no signs of a malignant course of hypertension no signs of cardiac failure or sympto matic hypertension Three patients have undergone sur gery In case 7 a bilateral nodular adrenal hyperplasia was revealed The hyperplasia mostly included zona glo merulosa The gland with the greatest changes was removed and the blood pressure is unchanged (6 months post operatively) Case 17 revealed a left sided adrenal ade noma The adenoma consisted of zona glomerulosa tissue the zona glomerulosa in the other adrenal gland was hypoplastic Blood pressure is normalizing after three months of observation In case 47 a left sided solitary adrenal adenoma was found Biopsy of the adrenal out side the tumor showed cortical atrophy Blood pressure became normal a few days after surgery

METHODS

An indwelling needle was placed in an antecubital vein Coagulation in the needle was prevented by flushing it with small amounts of Na-citrate (3 8) Immediately before blood samples were obtained 3 ml of blood was drawn and discarded to avoid citrate contamination from the needle Subsequently blood samples were drawn into two disposable syringes of 10 ml each containing 1 ml 3 8 Na-citrate The blood was transferred to a sili conized 50 ml Erlenmeyer flask immersed into ice water Within one hour the blood was centrifuged at 0 C 3000 rpm for 15 min The plasma was separated and kept at − 20 C until renin activity was determined

The PRA was determined by the method of Boucher et al with the modifications we have described (19) (coefficient of variance ± 11 8) The results are ex pressed as ng angiotensin/10 ml plasma 4 h incubation In addition to the blood obtained for renin assay another 5 ml of blood was drawn into a dry syringe for deter mination of colloid osmotic pressure (COP) plasma so dium and plasma potassium COP was recorded in an electronic osmometer for quick direct measurement of small samples as described by Hansen (13) The results are expressed in cm H O Confidence limits 95 = ±0 5 mm Hg Plasma volume in standing position as percent of value in supine position is determined as

$$\frac{\text{COP supine} \times 100}{\text{COP standing}}$$

Plasma sodium and potassium were determined by flame photometry Aldosterone secretion rate was determined by

Table II *Normal control on liberal salt intake The standing values are averages of 20 and 30 min samples*

Liberal salt intake	PRA Supine (mean±S E M)	PRA Standing (mean±S E M)	COP Supine (mean±S E M)	COP Standing (mean±S E M)	Standing plasma vol in % of supine (mean±S E M)
?? persons 17 males 5 females Range 18–67	15±2 Range 0–36[a]	34±2 Range 15–60	34 8±0 6	41 2±0 7	84 62±0 96

[a] Two persons showing 0

the double isotope method described by Kliman and Peterson (15) The determinations were performed by Dr J Jacobsen Med Dept. B Rigshospitalet. The aldosterone secretion rate was determined on the same day as PRA. Postural studies were made in all patients and 2_ normal controls on normal free salt intake all experiments being carried out between 8 a.m and 11 a.m When the patient had been in a supine position for 45 min the first blood sample was obtained as described previously (20) Knowing that a basal PRA level is obtained in normal subjects after this period of recumbency it was shown that the same applied to essential hypertensives by determining the values of PRA after 45 min and 7 h of recumbency in five hypertensive patients In no case did the difference between the two determinations exceed the 95% confidence limit of the method. The patients were asisted to a standing position and permitted to take a few steps occasionally Blood samples were obtained 5 10 20 and 30 min after the standing position had been assumed In all hypertensives the blood pressure was measured several times at the end of the period in supine position The mean of these determinations forms the mean blood pressure (diastolic pressure + 40% of the pulse amplitude) as shown in the Tables.

RESULTS

Normal individuals (Table II)

As described earlier by us (20) PRA reaches a plateau after 20 min of standing The increase in PRA takes place between 10 and 20 min in the upright position In all cases the 95% confidence limit is exceeded COP increases fast after the erect position is assumed on an average 80% of the total increase takes place within the first 10 min of standing The relation between ΔCOP and ΔPRA is described by the following regression line $y = 2\,8\,x + 1\,0$ $r = 0\,85$ $p < 0\,001$ (Fig 1)

Essential benign hypertensives (Table III)

The PRA in supine position is 25 ± 4 (S E M) which is significantly higher than in normal individuals ($t = 2\,23$ $n = 36$ $0\,05 > p > 0\,025$) In two patients (cases 30 and 32) PRA was above normal range but as in the other patients there were no signs of an accelerated or malignant course of hypertension Mean PRA in standing position was 36 ± 4 which is not significantly different from normal individuals ($t = 0\,44$ $n = 36$ $0\,70 > p > 0\,60$) No patients were clearly outside the normal range in the upright position As shown in Table III there is no PRA increase in six patients (cases 2 10 18 27 30 and 32) In another six patients an increase in PRA does take place as in normal individuals (cases 9 11 15 21 22 and 36) while in two patients the PRA does not increase until after 30 min of standing (cases 3 and 46) The number of patients with a PRA increase exceeding the 95% confidence limit is significantly lower than in normal subjects ($0\,02 < p < 0\,05$) Table III demonstrates that all patients with eye background changes grade 0 show a significant rise in PRA while in only four out of 11 patients with grade I–III does PRA increase significantly The difference between these two groups is not significant ($0\,10 < p < 0\,05$) The rise in COP takes place as in normal subjects A plateau is reached after 10 min in erect position

Plasma volume in the standing position in per cent of supine plasma volume is $84\,0 \pm 1\,3$ which is not significantly different from normal ($t = 0\,41$ $n = 36$ $0\,60 < p < 0\,70$)

Aldosterone secretion rate was determined in five patients The values were normal except in case 2 where the secretion rate was increased but the corresponding PRA was in the upper normal range as well

Essential malignant hypertensives (Table IV)

PRA in the supine position averages 148 ± 48 this is significantly higher than in patients with benign essential hypertension ($t = 2\,6$ $n = 18$

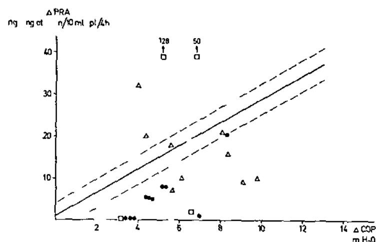


Fig 1 Correlation between increase in PRA and COP from resting values to 20 and 30 min of standing The regression line for normal controls with standard error of estimate (±1 Syx) is indicated Essential hypertensives with grade 0 = Δ Essential hypertensives with grade I–III = ● Essential hypertensives with grade IV = □

Table III *Essential benign hypertensives on liberal salt intake*

Indices 0=sample after 45 min supine 5 10 20 30=minutes after assumption of standing position Mean standing values are obtained as averages of 20 and 30 min values These mean values are used in the computation of standing plasma volume in of supine and standing PRA in of supine (PRAI)

Case no	Sex	Age (y)	PRA_0 / COP	PRA_5 / COP	PRA_{10} / COP_{10}	PRA_{20} / COP_{20}	PRA_{30} / COP_{30}	Mean stand PRA / COP	Stand pl vol in of supine	PRAI ( )	Mean BP / FH	Aldo secr rate (µg/24 h)
2	♂	50	36	36	44	40	36	38	94 1	106	158	294
			39 9	43 7	43 6	42 5	42 2	42 4			II	
3	♂	22	18	21	—	28	39	34	83 9	189	126	—
			37 0	42 6	42 3	43 1	45 1	44 1			0	
9	♂	53	4	5	10	13	14	14	77 8	350	96	—
			33 5	38 6	40 9	42 6	43 3	43 0			0	
10	♂	63	27	24	–	28	36	32	83 8	118	154	174
			34 8	38 6	39 6	41 8	41 1	41 5			II	
11	♂	43	36	36	28	48	53	51	81 7	142	212	—
			3(0	41 5	43 1	43 9	44 0	44 0			II	
12	♂	44	13	—	20	21	10	16	88 5	123	160	103
			42 3	46 6	46 6	47 6	47 9	47 8			II	
15	♂	47	16	—	15	31	28	30	82 3	188	170	—
			32 2	38 1	39 7	37 7	40 4	39 1			II	
18	♂	57	33	–	—	22	28	25	91 0	76	144	—
			36 7	42 0	40 6	40 5	40 1	40 3			II	
20	♂	54	0	–	0	—	13	13	73 7	—	132	89
			35 0	46 6	47 0	46 9	48 0	47 5			III	
21	♂	37	33	—	36	65	51	58	88 0	176	120	—
			35 3	43 4	41 0	39 3	40 9	40 1			0	
22	♀	55	3	0	0	19	—	19	79 3	633	132	—
			32 0	35 5	37 9	40 4	—	40 4			0	
27	♂	52	31	—	34	40	41	41	77 5	132	123	—
			37 1	—	44 9	46 6	47 8	47 2			I	
30	♀	43	56	—	55	61	68	65	83 3	116	164	113
			38 0	—	43 3	45 6	45 6	45 6			?	
32	♂	48	50	—	70	60	58	59	84 5	118	149	
			36 9	—	41 4	42 8	42 1	42 5			I	—
36	♂	47	16	—	19	35	25	30	85 5	187	137	—
			35 5	—	38 8	42 0	41 0	41 5			II	
46	o	22	29	—	31	36	49	43	88 4	148	1 4	—
			38 7	—	43 1	44 4	43 1	43 8			0	
Mean PRA±SEM			25 ±4					36 ±4	83 95 ±1 33			

able IV *Essential malignant hypertensives*

dices = Table III

ase o	Sex	Age (y)	PRA₀ COP₀	PRA₅ COP	PRA₁₀ COP₁₀	PRA₂₀ COP₂₀	PRA₃₀ COP₃₀	Mean stand PRA COP	Stand plasma vol in of supine	PRAI ( )	Mean BP FH	Aldo secr rate (μg 24 h)
1	♀	20	96	—	—	—	109	109	—	113	180	—
			—	—	—	—	—	—			IV	
3	♀	33	47	50	60	—	44	44	80 4	105	146	—
			30 5	34 5	36 3	38 6	37 1	37 9			IV	
1	♀	0	210	240	270	338	209	283	89 0	135	180	700
			34 6	39 0	40 1	40 0	37 8	38 9			IV	
5	♂	45	245	—	270	—	295	295	82 0	1 0	138	518
			30 7	—	33 9	—	37 5	37 5			IV	
Mean PRA ± S E M			148 ±48					183 ±63	83 8 ±2 64			

02 > p > 0 01) In three patients PRA is above normal range in the supine as well as in upright position The rise in PRA on assuming the standing position is not significant in any case The increase in COP takes place normally and the plasma volume in the erect position in percent of the supine value corresponds to that of normal subjects (t = 0 29 n = 24 0 80 > p > 0 70)

Aldosterone secretion rate was determined in two patients both values being clearly increased

Patients with signs of primary hyperaldosteronism (Table V)

PRA in the supine position averages 6 ± 2 which is significantly lower than in patients with essential hypertension (t = 4 08 n = 21 p < 0 001) Yet all values are within the range of essential benign hypertension PRA in the upright position averages 13 ± 4 this is significantly lower than in essential benign hypertension (t = 4 12 n = 21 p < 0 001) All values are within the range of essential benign

Table V *Patients with signs of primary hyperaldosteronism*

Indices = Table III

Case no	Sex	Age (y)	Salt intake mEq Na mEq K+	PRA₀ COP	PRA₁ COP	PRA₊₀ COP₂₀	PRA₃₀ COP₃₀	Mean stand PRA COP	Stand pl of in of supine	PRAI ( )	Mean BP FH	Plasma Na Plasma K
17	♂	53	80	10	6	35	23	29	74 2	290	18	146
			70	35 1	43 5	47 8	46 8	47 3			II	2 8
17	♂	53	80	0	0	4	—	4	8 6	—	141	147
			70	31 0	36 4	37 5	—	37 5			II	3 2
7	○	55	Liberal	18	30	26	26	26	80 3	145	157	143
				34 0	38 6	41 7	4 9	4 3			II	3 2
25	∩	53	Liberal	4	11	15	11	13	83 6	3 2	136	143
				30 4	33 4	37 1	35 6	36 4			I	3 7
25	♀	53	Liberal	6	0	0	12	6	90 5	100	—	142
				33 0	36 6	35 0	37 4	36 4			I	3 3
43	♀	43	80	4	11	10	4	7	90 7	175	14	144
			70	36 1	37 9	39 4	39 7	39 6			I	3 8
47	♀	43	80	0	5	6	5	6	90 4	—	132	145
			70	4 7	44 8	47 1	47 4	47 3			II	1 8
Mean PRA ± S E M				6 ±				13 ± 4	84 6 ±2 4			

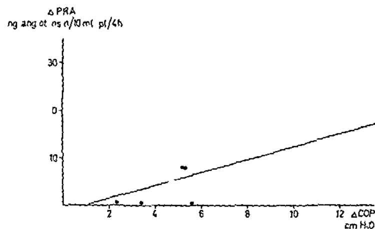


Fig Correlation between increase in PRA and COP from resting values to 0 and 30 min of standing Essential hypertensives with grade I–III = ● Primary hyperaldosteronism = O

hypertension In two patients cases 25 and 43 the postural increase in PRA is not significant

The plasma volume in the erect position in per cent of supine position is 84 6 ± 2 4 which is not significantly different from normal subjects (t = 0 08 n = 24 $p > 0\ 90$)

Fig 1 depicts the relationship between ΔCOP and ΔPRA from supine to plateau values for all hypertensives studied It is seen that most of the values are below the regression line of normal subjects and outside the standard error of estimate ($\pm S\ y\ x$) Correlation coefficient for hypertensives is $r = 0\ 09$ signifying no correlation In Fig 2 values deriving from patients with mild hypertension (grade 0) and severe hypertension (grade IV) are omitted The rest of the values demonstrate a pronounced linear correlation between ΔCOP and ΔPRA The regression line is $y = 1\ 4x - 1\ 2$ ($r = 0\ 68$) The correlation is significant ($t = 5\ 7$ $n = 32$ $p < 0\ 001$) This correlation line of hypertensive patients has a slope which differs significantly from the slope of the regression line of normals ($t = 4\ 0$ $n = 64$ $p < 0\ 001$) The ordinate of the y axis intersection of the regression line is not significantly different from zero ($t = 0\ 63$ $n = 32$ $0\ 50 < p < 0\ 60$) Fig 3 demonstrates a scatter plot of corresponding values of supine PRA and standing PRA in percent of supine (PRAI %) in normal subjects as well as in hypertensives The values describe a clear curvilinear correlation and depict only one population

DISCUSSION

In our group of essential benign hypertensives the mean values of PRA in the supine position are significantly higher than for normal subjects and the range is greater This finding is in accordance with Meyer et al (18) while Helmer (14) and Pickens et al (21) have found lower PRA values in similar groups Greco et al (11) Brown et al (1) and Weinberger et al (23) do not find values significantly different from normal individuals

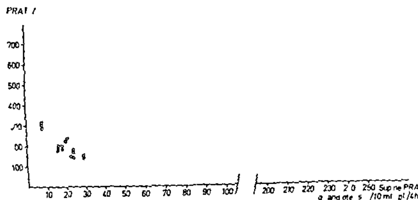


Fig 3 Correlation between PRAI (PRA in standing position in per cent of supine value) and the corresponding supine PRA value Normal controls = O All hypertensives investigated = ●

hese discrepancies may be due to different ratios f mild to severe hypertension in the materials he racial composition may also be of significance

The malignant hypertensives have an increased supine PRA compared with patients with ssential benign hypertension in correspondence ith data from other investigators (2 11 12 14 8 21 and 23)

The group of patients with signs of primary yperaldosteronism show a mean PRA significantly ecreased compared with essential benign hyper ives The values range from zero to normal this corresponds to the findings of others 3 4 5 and 6) in clearcut cases of primary hyper ldosteronism In all hypertensives the plasma olume depletion due to changes in posture from e re umbent to the standing position is within normal range unlike the findings of Eisenberg d Wolf (9) who find an increased extracellular volume contraction in hypertensive patients

Other investigators have shown a suppressed of PRA to the assumption of upright pos ture in a fraction of essential hypertensive pa tients While Warter et al (22) and Weinberger et al (23) as we do find a suppressed response in ents with high as well as low PRA Gunnels al (12) and Creditor and Lo chky (7) only find suppression in patients with low PRA Kuchel et al (16) by studying renin stimulation with diaz oxide demonstrated that patients not stimulated by th drug have a decreased mean PRA compared with other essential hypertensives Several of these nonresponding patients have undergone surgery and often no adrenal adenoma has been found

In our investigation the increase of PRA in hyp rtensives on assuming the standing position is evaluated in different ways

1 By indicating PRA in the erect posture in relationship to normal range In this way no significant difference between normals and patients with benign essential hypertension and primary hyperaldosteronism can be found

2 By indicating whether PRA response on as suming head-over feet position exceeds the 95 confidence limit of the method By using this criterion quite a number of hypertensives are distinguished from normals although not all

3 By studying the relationship between supine PRA values and PRAI% This relationship dem onstrates that normal subjects and hypertensive patients of all three types form one population In this manner no pathological response is dis closed

It is remarkable that PRAI% of high supine values is very close to 100% even in normals (Fig 3) accordingly the insignificant PRA re sponse to the erect position in the three cases of malignant hypertension is not clearly pathological

4 By studying the relationship between ΔCOP and ΔPRA Con idering ΔPRA in relation to hemoconcentration (expressed as ΔCOP) due to changes in posture (Fig 1) it is seen that the great majority of the patients increase their PRA less than do normals with the same degree of hemo concentration

However if the group of patients with fundo scopic changes of grades I–III is studied a sig nificant linear correlation is shown The correla tion line is clearly dissociated from the normal line by having a decreased slope

By excluding patients with fundoscopic changes of grade 0 and grade IV the group formed is more homogeneous as to degree of severity The fact that this group shows a uniform suppression in PRA response to postural changes does not eluci date the mechanism of the response In the present state of our knowledge the suppression may as well be the result of the hypertensive state as it may be an indicator of a hemodynamic dysfunc tion leading to hypertension Being uniform in degree of severity the patients of this group (grades I II III) may be divided into two sub groups

One subgroup is made up of patients with signs of primary hyperaldosteronism Three patients were submitted for surgery Two were classical cases of Conn s syndrome One patient revealed a nodular adrenal hyperplasia which has been described earlier in patients showing all features of primary aldosteronism (3 8) The remaining two patients also meet the requirements of primary hyperaldosteronism but they have not undergone surgery for private reasons

In the other subgroup it is reasonably assumed that the majority of the patients do not have primary hyperaldosteronism indicated by a normal to high PRA in the supine position There is general agreement that cases of normokalemic primary hyperaldosteronism do not have PRA in this range (3 4 5 and 6) In five of the patients

Table I *Results of tests on haemostatic function*[a]

Date	Bl T (sec) Ivy	Platelets (per mm) Feissly Ludin	Thrombelastogram *r* (min) Hartert	*k* (min) Hartert	*ma* (mm) Hartert	P T (sec) Quick	T T (sec)	Coagulation factors activity I (mg) Claus	II	V	VII	VIII	IX	X
									One stage methods plasma			of normal		
Case 2														
12 3 63	180	228 000	14	3	64	16 1	11 3	500	80	35	50			100
23 9 64	143	1 0 000	19	9	35	11 7	10	370						
21 7 67	172	157 000	23	10	42	12 0	9	390						
Case 3														
13 1 58	780	215 000					10 4		65	1?0	100			55
5 2 6?	480	244 000	25	5	65	11 8	12 5	660				41	71	
5 10 62	>900	356 000	17	8	56	13 4	13 6	490				19		
27 4 64	696	268 000	17	7	56	14 2		325	100	100	100	30	100	100
26 9 66	>900		16	9	45	12 7		620	100	110	100	17	115	1?0
4 3 68												11		
Case 4														
27 10 61		110 000				14 7	<8	370						
29 2 68	<60	84 000	24	14	40	15 4	8 2	385		86				
Case 5														
22 6 66	180	52 000	30	18	33	17 9	12 2	130	75	30	60			50
8 7 66		169 000	20	18	30	15 3	14 2	180	80	40	60			70
26 7 66		110 000				15 8		580	90	110	90			60
13 2 67	252	100 000	19	11	60	14 9	12 4	860	60	95	75			
Normal values (Mean ± S D)	117 ± 69	2 5 ± 0 5 10	18 0 ± 2 0	10 0 ± 2 2	47 0 ± 3 0	13 4 ± 1 4	15 ± 2 1	3 0 ± 40	Normal range 65–150					

[a] Bl T bleeding time P T = prothrombin time T T = thrombin time
case 2 fibrin split products were absent in ca e 3 no anticoagulant against factor VIII was found
ll tests performed by *Dr J J Veltkamp*)

with a planimeter The base line was not obtained from a double sector cell but extrapolated from the horizontal part of the Schlieren curve in front of the fastest peak as it appears at the beginning of sedimentation The relative percentage of a fraction is obtained in this way by putting the total surface at 100 Total protein and total macroglobulin are also given as absolute values which were calculated from the measured surfaces with the usual formula (27) A value of 0 000188 ml/mg for the refractive increment was calculated from an ultracentrifuge run of a purified IgM preparation the protein content of which was determined by the Kjeldahl method The macroglobulin content obtained by this procedure includes α_2 globulin as well as any normal heterogeneous IgM which may be present The contributions made by these components were disregarded assuming that their variation during follow up studies was negligible In one case of IgA paraproteinaemia (case 5) the level of the abnormal protein could be calculated by the same method since this component was mainly present in the form of fractions >7 S The contribution of 7 S IgA paraprotein was disregarded in this case Measurement by ultracentrifugation was preferred to Mancini s immunodiffusion method because this may give erroneous results when levels of different paraproteins have to be compared (14) The ultracentrifuge method gave a standard deviation of 0 03 g/100 ml for the single measurement of the macroglobulin peak

Viscosity was measured with an Ostwald viscosimeter using undiluted serum at a temperature of 37 C Corrections for specific weights were not applied since they were within the experimental error

Methods used in blood coagulation studies are mentioned in Table I For further references see Hensen and Loeliger (11) and Loeliger et al (19)

Immunofluorescence studies on bone marrow in case 5 were performed by the direct method using monospecific fluorescent conjugates and concentrated suspen ions of washed cells instead of smears (9)

CASE REPORTS

Case 1

S a 56-year-old man was referred to the hospital in September 1963 because of haemolyti anaemia which did

not sufficiently respond to prednisone treatment Physical examination revealed pallor petechiae on the lower legs peripheral oedema and a slightly enlarged spleen. The eye grounds did not show any haemorrhages Laboratory investigations haemoglobin 7 1 g/100 ml reticulocytes 0.. leucocytes 6.00 per mm with a normal differential count, platelets 240 000 per mm The blood smear showed rouleaux formation of the erythrocytes The ESR was 133 mm after one hour Total s rum bilirubin was 1 3 mg/100 ml with increased indirect reacting bilirubin The Bence Jones reaction in the urine was negative Results of all other routine laboratory tests were normal The cytolo_ic picture of the bone marrow smear revealed an increase in lymphocytic elements and erythroid hyperplasia The direct Coombs test was positive tests for cold agglutinins were negative A Cr^{51} red cell survival gave a T / of 8 days and scanning demonstrated an increase in splenic sequestration of erythrocytes The total protein was 7 4 g/100 ml Agar and immunoe ectrophoresis of serum revealed an IgM paraprotein In the ultracentrifuge a 19 S macroglobulin content of 23 of the total protein was measured A diagnosis of Waldenstrom s macroglobulinaemia with haemolytic anaemia was made and treatment with chlorambucil in a dose of 4 mg per day was instituted, while prednisone was continued in a dose of 40 mg/day Three months later there was a subjective improvement. The direct Coombs test was still found positi e The course of other relevant laboratory investigations and of the therapy during the case history is represented in Fig 1 After a nine months course of treatment with chlorambucil the haemoglobin had hardly chang d, but a decrease in macroglobulin content of the serum was found. In November 1964 the prednisone was discontinued. One month later laboratory investigations disclosed a decrease in haemoglobin from 10 to 7 9 g/ 100 ml The cytologic picture of the bone marrow smear showed marked erythroid hyperplasia After reinstitution of prednisone the haemoglobin rose gradually to 10 8 g 100 ml In December 1964 the chlorambucil was discontinued for several months because of a low white cell count. In November 1965 the patient complained of a tumour in the left inguinal fossa. Physical examination re ealed unilateral inguinal lymphadenopathy Cytologic examination of an inguinal lymph node revealed reticulum cell sarcoma There was an increase in lymphocytic elements mast cells and plasma cells in the bone marrow The titre of the d rect Coombs test had decreased. Ultracen r fugation of the s rum showed that the macroglobulin level had risen. A chest X ray and an abdominal lymphangiogram were normal In January 1966 splenectomy was performed in an attempt to treat the haemolysis The patient died two weeks later of pulmonary embolism

Case ?

B a 70-year-old man was admitted to the hospital in January 1963 because of fatigue and haemolytic anaemia He was treated with prednisone in a dose of 0 mg per day On this therapy his general condition deteriorated and he was referred to the University H spital in Le den in March 1963 for further treatment. On admittance pallor and jaundice were not ced the liver was felt 3 cm below the right costal margin and the spleen 4 cm below the

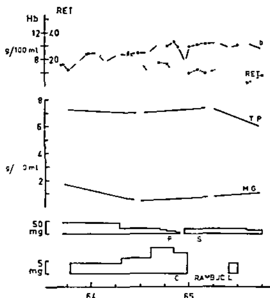


Fig 1 Clinical course of case 1 (S) Hb = haemoglobin. RET = reticulocytes T P = total protein M G = macroglobulin Time scale on abscissa

left costal margin. The temperature was 39 6 C Funduscopic examination revealed no abnormalities Laboratory investigations haemoglobin 3.2 g/100 ml erythrocytes 0 8 mill per mm³ reticulocytes 49 1 leucocytes 8 00 per mm with a normal differential count thrombocytes 2?5 000 per mm The blood smear showed rouleaux formation of the red cells marked anisocytosis poikilocytosis, polychromasia and many normoblasts The ESR was 176 mm after one hour The total serum bilirubin was 5 mg/ 100 ml with increased indirect reacting bilirubin, and the LDH was 800 units The urine contained excess of urobilinogen the reaction to Bence Jones protein was negative The cytolog c picture of the bone marrow smear showed a marked erythroid hyperplasia and an increase in mast cells The direct Coombs test was positive the cold agglutinin test was negative The total serum protein was 8 5 g/100 ml Agar and immuno-electrophoresis of the serum revealed an IgM paraprotein which proved to be a cryoglobul n In the ultracentrifuge a ma-roglobulin content of 34 of the total serum protein was measured In estigation of haemostatic functions revealed some abnormal t es see Table I X ray examination of the skeleton showed no abnormal t es A diagnosis of Waldenstrom s macroglobulinaemia w th haemolytic anaemia was made Four units of packed red cells were transfused and the dosage of prednisone was increased to 60 mg per day Thereafter his condition improved rapidly The course of the disease was complicated by an infection with Salmonella paratyphi D which was successfully treated with ampicillin By the end of March 1963 the haemoglobin and reticulocyte count values had cons derably improved. The total serum bilirubin was 1 06 mg 100 ml Treatment with chlorambucil n a dose of 6 mg per day was started and the prednisone dosage was decreased to 30 mg per

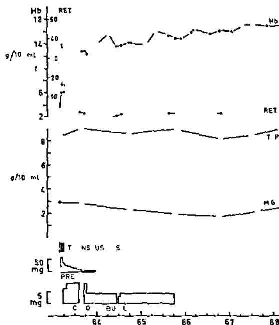


Fig 2 Clinical course of case 2 (B)

day Examination of a bone marrow smear showed focal erythroid hyperplasia a large number of mast cells an increase in lymphocytic cells sometimes seen in clumps and an increase in plasma cells For the course of labora tory investigations and of therapy see Fig 2 Five months later the patient was feeling well The titre of the direct Coombs test had decreased and was found to be negative O tober 1965 The treatment with chlorambucil was ued until October 1965 but the prednisone dosage gradually decreased and ultimately stopped by Decem 1963 During 1964 and 1965 the patient was free from symptoms In October 1966 the patient was found to be in a good general condition and a physical examination revealed no abnormalities In January 1967 the cytology of the bone marrow showed a decrease of lymphocytic cells compared with four years previously At the time of writing this article 28 months after the cessation of the chlorambucil treatment the patient is still feeling well

Case 3

G a 74 year-old man was first seen in 1958 because of a two years history of nosebleeds and chronic bronchitis Physical examination revealed no abnormalities The haemoglobin was 13 1 g/100 ml erythrocyte count 4 1 mill per mm reticulocytes 2 6 thrombocyte count 215 000 per mm ESR 34 mm after one hour total serum protein 8 6 g/100 ml Investigation of haemostatic func tions showed a prolonged bleeding time see Table I Other routine laboratory tests were normal Roentgenologi cal examination of the chest showed no abnormalities In April 1961 he visited the outpatient department complain ing of frequent nosebleeds The haemoglobin value was 9 6 g/100 ml The patient was found to have coronary sclerosis and congestive heart failure Treatment consisted of digitalis diuretics and oral iron In February 196 he had many severe nosebleeds necessitating blood trans- fusions Laboratory investigations revealed a low activity of factor VIII (see Table I) A tentative diagnosis of vascular haemophilia (Willebrand s disease) was made although a family study gave negative results In March 1962 he was admitted to the hospital because of melaena, probably caused by a sliding hernia of the oesophagus.

A haemoglobin value of 6 6 g/100 ml was found. In 1963 he had no complaints but in 1964 he again suffered from intermittent nosebleeds and fatigue On physical examination no abnormalities were found Laboratory in vestigations revealed haemoglobin 13 g/100 ml leucocyte count 5100 per mm^3 serum calcium 9 9 mg/100 ml and total serum protein 6 9 g/100 ml The direct Coombs test was negative and the reaction for Bence Jones protein in the urine was also negative An IgM paraprotein was found in the serum by agar and immuno-electrophoresis. On ultracentrifugation it appeared that the macroglobulin fraction amounted to 10 % of the total serum protein. Examination of a bone marrow smear revealed the pre ponderance of plasma cells many of which contained large Russell bodies A roentgenological examination of the skeleton showed no abnormalities A diagnosis of Waldenstrom s macroglobulinaemia was suspected. For the course of the relevant laboratory investigation and of the therapy see Fig 3 In December 1964 ultracentrifugation showed that the macroglobulin level was almost normal A treatment with chlorambucil was started in May 1965 During 1966 the patient had only a few nosebleeds. At the time of publication the patient is still on chlorambucil treatment He has few and small nosebleeds The haemo- static functions have not improved

Case 4

R a 69 year-old man was admitted to th hospital in August 1961 with a six months history of nosebleeds, bleeding gums anaemia and some blurring of vision

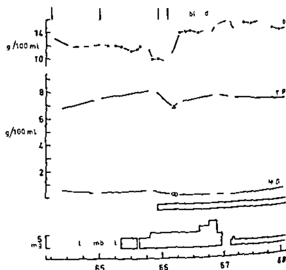


Fig 3 Clinical course of case 3 (G)

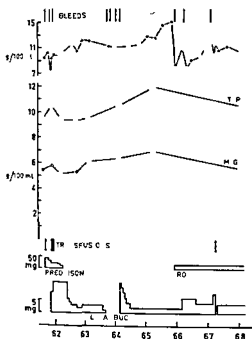


Fig. 4. Clinical course of case 4 (R.).

(clinical data made available by Dr. J. F. L. Fontein). A month prior to his admission he had been examined by an opthalmologist who found a distension of the retinal veins with multiple haemorrhages. The visual acuity was 10/10 in the left and 5/10 in the right eye. On physical examination the liver was felt 3 cm below the right costal margin. There was no splenomegaly. Laboratory data: haemoglobin 9.5 g/100 ml, reticulocytes 1.7%, thrombocytes 130 000 per mm³ and ESR 135 mm after one hour. The results of other routine laboratory tests were essentially within the normal range. Examination of a bone marrow smear revealed focal erythroid hyperplasia and an increase in lymphocytic cells. The total protein was 9.5. An IgM paraprotein was detected in the serum by agar and immuno-electrophoresis. A macroglobulin content of 57% of the total serum protein was measured by ultracentrifugation of the serum. A diagnosis of Waldenström's macroglobulinaemia was made. Two units of blood were transfused and a treatment with prednisone in a dose of 40 mg per day was instituted. The patient was readmitted in October 1961 because of severely bleeding gums and dizziness, possibly of vestibular origin. Now the spleen was felt 2 cm below the left costal margin. The haemoglobin was 7.2 g/100 ml. The results of the tests on haemostatic functions are presented in Table I. Six units of blood were transfused and treatment with chlorambucil in a dose of 8–12 mg was instituted. For the course of relevant laboratory tests and of the therapy see Fig. 4. In March 196[illegible] the patient had fewer episodes of bleeding from the nose and the gums and there was no splenomegaly. The prednisone was discontinued. During 1963 the patient was feeling well. The chlorambucil treatment was discontinued in September 1963. From October 1963 until March 1964 the patient suffered from intermittent bleeding from the nose and the gums. A treatment with chlorambucil in a dose of 1[illegible] mg per day was reinstituted. In May 1964 the patient had no complaints of bleeding. He remained free from symptoms until December 1965 when he again experienced severe bleeding from the gums and epistaxes, accompanied by a strong decrease in haemoglobin. A treatment with iron was instituted. Because of persisting bleeds the dose of chlorambucil was increased in March 1966. From May 1966 until March 1967 the patient only had intermittent very small nosebleeds. On March 8, 1967 an operation performed because of a basalioma was complicated by haemorrhages. In September 1967 the patient complained of a total loss of vision of the right eye. He was seen by his opthalmologist, who found an ablatio retinae. Funduscopic examination also revealed hardly any improvement of the left eye: distended retinal veins and scattered haemorrhages were still present. On a return visit in January 1968 the patient had no further complaints. Physical examination revealed no abnormalities. Laboratory data in February 1968: The ESR was 139 mm after one hour, serum calcium 8.9 mg/100 ml, serum uric acid 7.4 mg/100 ml. The Bence Jones reaction was negative. The direct Coombs' test was negative, cold agglutination was also negative. Investigation of haemostatic functions: see Table I. The patient is maintained on 4 mg chlorambucil daily.

Case 5

L., a 75-year-old woman, was admitted to the hospital in June 1966 because of severe epistaxes and diminished consciousness. Pallor was noted but further physical examination revealed no abnormalities. Laboratory investigations: haemoglobin 5.5 g/100 ml, erythrocyte count 1.6 mill. per mm³, reticulocytes 6%, leucocyte count 7100 per mm³, platelets 52 000 per mm³, ESR 16[illegible] mm after one hour, serum calcium 12.4 mg/100 ml. The Bence Jones reaction in urine was negative. The remainder of the routine laboratory tests were normal. Cytology of the bone marrow smear revealed a replacement of normal cells by myeloma cells. The nucleus of many of these myeloma cells was centrally placed and showed large nucleoli. Immunofluorescent studies of these cells (performed by Miss H. R. E. Schuit) showed the presence of a monoclonal IgA protein of the kappa type. The direct Coombs' test was negative. The total serum protein was 10.4 g/100 ml. Agar and immunoelectrophoresis revealed an IgA paraprotein. Ultracentrifugation showed the presence of a large amount of IgA with sedimentation constants of 8.5, 10 and 1[illegible] Svedberg units (S). These protein components accounted for 69% of the total protein. Investigation of haemostatic functions showed several disturbances: see Table I.

Roentgenological examination of the skeleton showed no abnormalities. A diagnosis of multiple myeloma was made and treatment with blood transfusions, prednisone in a dose of 40 mg per day and alkeran in a dose of 5 mg per day was started. A total of 70 mg alkeran was administered. The clinical condition of the patient improved markedly within a month. The haemoglobin value had risen to 13.8 g/100 ml, serum calcium was 8.9 mg/100 ml. Ultracentrifugation demonstrated a decrease in IgA con-

chlorambucil was started the patient was free from symptoms for almost a year A relation of the paraprotein to the bleeding does not seem likely since the level was always very low and the spontaneous decrease in 1964 was not attended by a clearcut improvement It should be noted that factor VIII deficiency in macroglobulinaemia was also found by Nilehn (24) who could not show a direct effect of the paraproteins on this deficiency in his patients

In case 4 the chlorambucil therapy seemed to have a favourable effect on the bleeding tendency *since the bleeding returned on several occasions* when the drug was stopped or lowered This phenomenon seemed unrelated to the presence of the paraprotein which was always at a very high level It should be stressed however that a causal relationship between the therapy and the disappearance of bleeding cannot be proved in view of the irregular occurrence of such bleedings

The clotting defects in Waldenstrom s macro globulinaemia have been attributed to coating of platelets by the macroglobulin or to various effects on the vessel wall or on clotting factors (13 18 23 24) although the observations of Perry (26) did not confirm the latter view

In the myeloma case there was an influence of therapy on the haemostatic functions which seemed be related to the level of the paraprotein Increased serum viscosity may have caused the bleeding tendency here but an effect of paraproteins on the clotting mechanism has also been postulated (7 12 32)

Viscosity

Bleeding tendency has been attributed to increased serum viscosity caused by the pathological macro globulin (see Table II) In case 3 the viscosity was not different from that of normal serum while in case 4 the viscosity was consistently high as could be expected from the paraprotein level Treatment with chlorambucil had no effect on viscosity in the latter case although the bleedings decreased considerably except in the retina Mechanical con siderations would lead one to assume that the retinal vessels are a very sensitive target organ for deviations caused by a high blood viscosity Our observations in case 4 do not exclude this pos sibility which calls for further investigation The serum viscosity was high before treatment and decreased afterwards in case 5 This case also demonstrates that a strongly increased serum viscosity need not be caused by a macroglobulin of the IgM type so that viscosity measurements cannot always support the diagnosis of Waldenstrom s macroglobulinaemia

General discussion

The study of our cases demonstrates that alkylating agents do not always have an effect on the paraprotein level although this has often been observed Underdosage as a cause of failure to affect the paraprotein level seems to be unlikely *because of the existence of drug induced leucopenia* in all patients The clinical effect of therapy may be attributed to prednisone in cases 1 and 2 and cannot be conclusively demonstrated in cases 3 and 4 A clearcut correlation between para protein level and clinical symptoms seems to be absent in all cases

The conflicting statements concerning the effect of the paraprotein on various symptoms of Wal denstrom s macroglobulinaemia may reflect the individuality of these abnormal proteins Individual differences can be found in electrophoretic mobility solubility and L or H chain type and offer the possibility to make monospecific antisera (16) These differences may also be found with regard to interference in clotting mechanism etc and might also explain why for example an increased serum viscosity does not always result in a bleeding tendency (cf 10) It is therefore very possible that in some cases the paraprotein level is indeed a useful parameter for following the course of the disease This however has first to be established by prolonged observation of the individual case *Before this has been done neither serum viscosity* nor paraprotein level are sure to give an indication of the clinical condition

The single myeloma case taken for comparison in this study seems to confirm this rule since the most important symptom the osteolysis became visible when the paraprotein was at its lowest level Alexanian et al (1) however found the myeloma protein level to be an important parameter of disease activity in a study of 82 patients

It may be inferred from our observations that the formation of an IgM paraprotein in Waldenstrom s macroglobulinaemia is a process not necessarily related to other symptoms of the disease This means that the view of the paraprotein formation as the primary defect implicit in many dis

sions may be questioned The production of clonal IgM rather is one of many symptoms expressing themselves in varying degrees without the underlying cause being known at present

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A COMPARATIVE STUDY CONCERNING THE STABILITY OF THE ANTICOAGULANT EFFECT OF ACENOCOUMAROL AND PHENPROCOUMON

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Abstract Two comparable groups, each of which comprised 41 patients, were treated with long acting phenprocoumon and short acting acenocoumarol, respectively for six months. When phenprocoumon was used, a significantly more stable anticoagulant effect was seen. This was reflected by a greater percentage of thrombotest values within the intended therapeutic zone ranging from 5 to 10% thrombotest activity inclusive Moreover there was a difference in the facility with which patients could be kept in the therapeutic range the number of alterations that had to be made in maintenance dose and control interval was less with phenprocoumon than with acenocoumarol.

The results obtained by long term treatment with anticoagulants to prevent cardiac infarction depend upon the intensity of this treatment (7 12) Also venous thromboses can be better prevented by an intensive treatment schedule (13) Though there are indications (2 3 5 6 11 14 15) that the long acting coumarin derivative phenprocoumon, will yield a more stable anticoagulant effect than other coumarin drugs, it is not known whether this will hold also if constantly low contents of vitamin K dependent factors are aimed at.

In this paper data are presented which were collected during intensive treatment with long acting phenprocoumon (Marcoumar®) and short acting acenocoumarol (Sintrom®) in two groups of patients during a period of six months The thrombotest values obtained in all patients and the ease with which the values to be aimed at could be realized with each of the drugs under study were analysed statistically

MATERIAL AND METHODS

Comparability of the two groups of subjects

A random selection was made of 110 subjects out of a group of patients who had been under prophylactic treatment with anticoagulants for more than one year These subjects were treated as outpatients with acenocoumarol They were classified according to the variations observed in thrombotest values during eight months prior to the study Thrombotest results were expressed as percentages of coagulation activity The mean and the standard deviation (S.D.) of the percentages of coagulation activity were calculated for each patient. S.D. varied from ±1 8 % in patients with stable hypocoagulability to ±5.6 in a very unstable patient. Because of these large differences in stability it was decided to make a paired comparison experiment. Fifty five couples were formed so that the difference in S.D. between partners was as small as possible Beginning with January 1967 acenocoumarol was replaced by phenprocoumon for one of the partners in each of the 55 couples, the choice being made by drawing lots. The stability of the anticoagulant effect of the two drugs was compared in the period between April 1 1967 and October 1 1967 The period of comparison was not started before April 15 because the use of a new drug may at first influence the accuracy with which the prescribed dose is taken

Twenty-six patients dropped out between the moment of patient selection and the end of the period of study Two patients appeared to be wrongly classified one of them took phenprocoumon before the investigation, while the other was not an outpatient. Six patients died. Eight patients were taken to hospital for a complicating disease In four patients control was taken over by another thrombosis service In five patients treatment was stopped by their doctor who was not the thrombosis service specialist. One patient appeared to be resistant to acenocoumarol.

Because both patients dropped out in only a few couples, four new couples were formed in which the difference in S.D. prior to the study was not larger than in the former ones. In total, 41 couples remained for comparison Seven of these 82 patients have dropped out for a short time during the period of the investigation. In the remaining period, however at least six thrombotest determinations were done The group of patients who started taking phenprocoumon and the group of those who went on taking acenocoumarol were in close conformity with each other Mean ages were 58 7 and 60.6 years, respectively

Table III *Dosage scheme*

TT activity	Control interval	Dose
In therapeutic range 5–10	3 weeks	Unchanged
<5	≤1 week	1 or 2 days nil then changed or unchanged
≥11 to ≤15 following 2 controls in therapeutic range	3 weeks	Unchanged
>15% following 2 controls in therapeutic range	<3 weeks	Unchanged
2 of 3 subsequent control ≥11	<3 weeks	Changed

RESULTS

The stability of the anticoagulant effect of acenocoumarol and phenprocoumon in two groups of 41 patients each

After the formation of pairs of comparable patients and the substitution of phenprocoumon therapy for the acenocoumarol treatment in one of the partners of each pair (January 1967 until April 15 1967) a comparison was made between the thrombotest values obtained in each of two partners during the period from April 15 to October 15 1967 In the period of comparison 364 thrombotest determinations were made in the phenprocoumon group and 394 in the acenocoumarol group

The stability of the anticoagulant effect can be assessed by calculation of the standard deviation of the thrombotest percentages of each patient during the period of comparison There was a high degree of stability (S D ≤3 %) in 31 of 41 patients taking phenprocoumon but only in 19 of 41 patients taking acenocoumarol An unstable anticoagulant effect (S D ≥4 6) was seen three times with phenprocoumon and 11 times with acenocoumarol (Fig 1)

In the phenprocoumon group 78 3 % of thrombotest activities were found to fall within the therapeutic zone aimed at ranging from 5 to 10 % inclusive This figure was 62 4 % in the acenocoumarol group A closer analysis of thrombotest values falling outside the therapeutic range was in favour of phenprocoumon (Table IV)

When a check was made as to the number of patients in whom all thrombotest results fell into the therapeutic range aimed at this appeared to be the case in 11 of 41 subjects treated with phenprocoumon and only in one of 41 subjects treated with acenocoumarol A very poor result of anticoagulant therapy with less than 50 % of thrombotests lying within the therapeutic optimum envisaged was seen only in nine patients of the acenocoumarol group (Fig 2)

If stability of adjustment in a group of patients treated with coumarin drugs is not satisfactory the number of controls and the number of times the dose has to be changed will be greater than if a constant hypocoagulability has been achieved The number of times the dose had to be changed was 116 in the acenocoumarol group as compared with 58 in the phenprocoumon group (Table V)

The dosage range of a patient i e the difference between the maximum and minimum dose prescribed during the period of comparison was on the average 0 3 mg when phenprocoumon was used as compared with 0 7 mg for acenocoumarol These averages have been corrected for the difference in action potency 4 mg acenocoumarol corresponding to 2 7 mg phenprocoumon (Table VI)

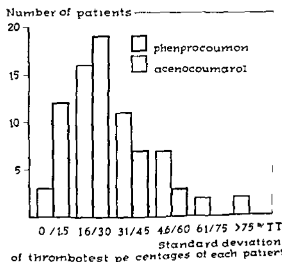


Fig 1 Stability of the anticoagulant effect when using phenprocoumon and acenocoumarol for six months

Table I *Comparability of the two patient groups in clinical respects*

	Pats changing to phenprocoumon	Pats continuing to use acenocoumarol
No of males	34	33
No of females	7	8
Average age	58 7	60 6
Indication for treatment		
Coronary insufficiency or infarction	27	32
Claudication or va cular graft	9	4
Various indications	5	5
Heart failure	5	7
Total no of pats	41	41

the sex ratio was nearly identical and so were the indications for treatment and the frequency of heart failure (Table 1)

As the pairs were formed on account of the stability of drug action during the months preceding the period of study an almost identical composition of the two patient groups with respect to the number of patients with stable and unstable levels of hypocoagulability the dose of acenocoumarol administered and the number of blood controls required could be expected and was in fact found (Table II)

Control of the anticoagulant effect

The effect of the coumarin derivatives used on blood coagulation was measured by means of the thrombotest ing to the prescription proposed by the manufac (Nyegaard & Co Oslo Norway) In the morning venous blood was taken in disposable polypropylene tubes with 3 8 trisodium citrate dihydrate as an anticoagulant in a ratio of 9 parts of blood to 1 part of citrate solution. The thrombotest determination was made within 5 hours after taking blood Determinations were made at least once every three weeks in each patient

Dosage and application of coumarin derivatives

The dosage of phenprocoumon 3 (1 phenyl propyl)-4-hydroxycoumarin (Marcoumar®) 3 mg per tablet, and acenocoumarol 3(alpha acetoxyl *p* nitrobenzyl) 4-hydroxy coumarin (Sintrom®) 4 mg per tablet was adjusted by one of us (W B) according to the scheme shown in Table III Patients were advised to take all of the dose prescribed at one time in the morning Beginning with January 1967 a thrombotest activity ranging from 5 to 11% was aimed at which was lower than in the preceding period

Table II *Comparability of the two patient groups as concerns the effect of anticoagulant administration in the period preceding the period of comparison (Jan 1 1966 to Sept 1 1966)*

	Pats changing to phenprocoumon	Pats continuing to use acenocoumarol
Standard deviation of T T activities in a pat		
Average	5 6	5 6
Range	1 8–12 3	1 8–12 6
No of pats with		
100 of controls between 6 and 20 T T activity	15	13
80–100 % of controls between 6 and 20 T T activity	13	14
<80 of controls between 6 and 20 T T activity	13	14
Dose of acenocoumarol (mg/day)		
Average	3 27	3 17
Range	1 0–6 0	1 5–5 4
Difference in maximum and minimum dose/pat		
Average	0 64	0 6[illegible]
No of controls/pat		
Average	11 2	10 5
Range	7–29	8–19

Table VI *Dosage and dosage range (difference between maximum and minimum dose per patient) using phenprocoumon and acenocoumarol*

	Use of phenprocoumon	Use of acenocoumarol
Mean dose/day (mg)	2.7	4.0
Mean dosage range in mg (difference between maximum and minimum dose/pat.)	0.2	0.7
Mean "corrected" dosage range in mg (correction for anti-coagulant activity 4.0 mg acenocoumarol = 2.7 mg phenprocoumon)	0.3	0.7

and were excluded from the statistical analysis if the differences between the members of each pair were smaller than 5%. In 25 of the remaining 35 pairs the percentage of controls within the therapeutic range was in favour of the phenprocoumon treated patients. This difference proved to be significant ($p = 0.0007$) (Table VII). An advantage of phenprocoumon partners was found in 17 of 20 pairs for percentages of T.T. values $\geq 15\%$, in 23 of 34 pairs for percentages $\geq 11\%$ and in 16 of 22 pairs for percentages $< 5\%$. All these differences were significant (Table VII).

3. *Dosage range and number of controls.* After exclusion of six couples in which one or both of the partners were treated for less than six months and of the pairs showing small differences, the phenprocoumon patients showed significantly smaller dosage ranges and less dose alterations than their acenocoumarol partners (Table VII).

Complications and toxicity

Despite the intensity of hypocoagulability during 479 months on therapy there were only 5 small bleedings and for these no specific treatment was required. Because of the rarity of bleeding events a comparison of this risk during treatment with acenocoumarol and phenprocoumon was not feasible. No toxic side effects were seen.

DISCUSSION

It had already been demonstrated by Borchgrevink (1) that an intensive treatment with oral anticoagulants in patients with angina pectoris reduces

Table VII *Significance of differences found in the stability of the anticoagulant effect and the ease with which this was attained in pairs of patients using phenprocoumon and acenocoumarol respectively*

	Total no. of couples compared	No. of couples: In favour of phenprocoumon treated partner	No. of couples: In favour of acenocoumarol treated partner	Undecided	p-value
Standard deviation of the percentages of T.T. activity/pat.	41	27	6	8	0.002
Percentages of T.T. values/pat. within the therapeutic range	41	25	10	6	0.0007
Percentage of T.T. values/pat. outside the therapeutic range					
Percentage ≥ 15 T.T. activity	41	17	3	21	0.003
Percentage ≥ 11 T.T. activity	41	23	11	7	0.007
Percentage < 5 T.T. activity	41	16	6	19	0.03
Corrected dosage range	35	27	5	3	0.00[illegible]
No. of dose alterations	35	24	5	6	0.001
No. of controls	35	18	10	7	0.07

Six couples of which at least one partner was treated for less than six months were excluded.
Differences considered to be too small.
p-value (observed level of significance) of Wilcoxon's two-sided signed rank test.

mortality and frequency of infarctions if this group is compared with patients on a less intensive treatment schedule According to Rozenberg et al (12) the occurrence of arterial thromboses was higher in patients with a desirable therapeutic range of 10–20% thrombotest activity than in patients treated more intensively A long term anticoagulant treatment after myocardial infarction effecting a profound and stable hypocoagulability (between 5 and 11 ′ thrombotest activity) yielded favourable clinical results according to Loeliger et al (7) and Meuwissen et al (8) If such an intense and stable hypocoagulability is aimed at without increasing the bleeding hazard too much a meticulous control of the patients and of the anticoagulant activity of the coumarin drug to be used is inevitable (4 9 10)

Although a more constant effect might be expected from the long acting drug phenprocoumon no data about the quantitative differences between this drug and acenocoumarol were available Nor was it known whether differences might remain if patients were treated intensively The differences found in this study are distinctly in favour of the long acting coumarin drug phenprocoumon

The use of the standard deviation of the percentages of thrombotest activity can be criticized be ~ ~ thrombotest values lower than 5% cannot , determined exactly However in practice the standard deviation turned out to be a quite suitable measure of the stability of the treatment for individual patien s and in this study the evaluation of the experimental results using other measures of stability like the percentage of T T values within the therapeutic range completely confirmed the conclusions based on the use of the standard deviation

The greater instability found with the use of acenocoumarol is almost certainly not due to a more frequent use of other drugs that might have interfered with the coumarin action Information obtained from the patients has given the impression that drug consumption in both groups was almost equal

ACKNOWLEDGEMENT

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Generic and trade names of drugs

Phenprocoumon Marcoumar Liquamar Falithrom.
Acenocoumarol Sintrom Nicoumalone

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Acta med scand Vol 186 pp 289-292 1969

MONOCLONAL GAMMOPATHY (IgG) AND CHRONIC ULCERATIVE DERMATITIS (PHAGEDENIC PYODERMA)

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Abstract Case report of a patient with chronic ulcerative dermatitis and monoclonal gammopathy No signs of myeloma could be detected The *M*-component was found to be of class *G* L-chain type *K* and subtype 3

Monoclonal gammopathy is usually found in multiple myeloma (in which case the protein fraction consists of molecules of class IgG IgA or IgD) and in Waldenstrom s macroglobulinaemia (in which case an *M*-component of the IgM class is present) *M*-components are being reported however with increasing frequency in various other diseases also (1 3 4 5 9) Skin diseases too can be associated with such a pathological protein fraction (6) In 1964 Rockl et al (7) described six patients with chronic ulcerative dermatitis in four of whom an *M*-component of the IgA class was found One of these four patients was suspected to suffer from multiple myeloma. Van der Sluis (8) described two patients and Meyers et al (6) one patient with chronic ulcerative dermatitis and an IgA *M*-component Danon et al (1) reported on four patients without specifying either *M*-component classification or clinical details There would seem to be a correlation between two rather infrequently occurring conditions namely ulcerative dermatitis and monoclonal gammopathy of the IgA class Degos et al (2) recently described a patient with this skin disease whose serum contained an IgG *M* component So far as we know this is the first and only patient described in the literature who showed the combination of chronic ulcerative dermatitis and IgG paraproteinaemia We observed a female patient with chronic ulcerative dermatitis (phagedenic pyoderma) whose serum likewise contained an IgG *M* component

CASE REPORT

Patient P G a woman aged 60 In the summer of 1966 this patient developed a painful ulcerative lesion of the left lower leg, which was refractory to conventional therapeutic measures A similar lesion subsequently developed on the right lower leg. At examination an ulcerative abnormality was found on the dorsal aspect of the left and the ventral aspect of the right lower leg There were no superficial varices. The condition was identified as superficially localized ulcerative dermatitis with phagedenic dissemination and formation of numerous small pustules at many sites along the slightly elevated margin The centre of the lesion showed coarse lamellar desquamation and crust formation, discontinuous at some sites (Fig 1)

No abnormalities other than these skin changes were found

Laboratory findings ESR 80 mm after 1 hour haemogram normal Urine normal normal blood sugar level liver function tests and electrolyte creatinine and urea concentrations. At paper electrophoresis of the serum a homogeneous protein fraction was demonstrated in the medium speed γ-globulin region. This was confirmed by agar electrophoresis (Fig 2 *a*) Immunoelectrophoresis (with horse anti human serum) showed that the M-component was of class IgG (Figs. 2 *b c*) With specific antisera the L-chains were of type *K* and the γ-chains (tested in immunoelectrophoresis and immunodiffusion experiments) of subtype $\gamma 3$ (Figs 2 *c d e*) Immunoelectrophoretic experiments showed diminished values for normal immunoglobulins IgG IgA and IgM

The finding of this M-component prompted an exhaustive study to establish the presence of multiple myeloma. Skeletal X rays disclosed no abnormalities apart from some mild arthritis deformans. Repeated examination of the bone marrow failed to reveal any abnormality (specifically no plasmocytosis) No Bence Jones protein was

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Acta med scand Vol 186 pp 293–297 1969

THE GROWTH HORMONE DEPENDENT SULFATION FACTOR IN SERUM FROM PATIENTS WITH VARIOUS TYPES OF DIABETES

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Abstract The growth hormone dependent sulfation factor is low in serum from diabetics There is no difference between the values obtained from obese and non-obese diabetics Nor is there any difference between the values obtained from recently diagnosed untreated cases and long term diabetics with severe diabetic angiopathy In non obese untreated diabetics a negative correlation obtains between the fasting serum sulfation factor and the serum glucose One hour after ingestion of glucose the sulfation factor falls In obese diabetics no correlation was observed between serum sulfation factor and serum glucose

In a series of publications beginning in 1924 Houssay and coworkers demonstrated the importance of the pituitary gland for carbohydrate metabolism They showed that hypophysectomized animals became extremely sensitive to insulin (15) that hypophysectomy alleviates the severity of pancreatectomy-diabetes (16) and that injection of pituitary extracts to partially pancreatectomized dogs induces a transitory diabetic state (17) In 1937 Young (38) working with intact dogs showed that injection of pituitary extracts repeated for a period of time resulted in a permanent diabetic state In 1949 Cotes et al (8) produced a diabetic condition in the adult intact cat by repeated injections of purified ox growth hormone thereby proving that the diabetes inducing agent in the pituitary extract was growth hormone These animal experiments have been confirmed in man by Luft (23) However their subjects treated with human growth hormone exhibited only a mild symptomless diabetes except for a group of hypophysectomized juvenile diabetics who developed a severe diabetic acidosis

Growth hormone has been thought to play a role in the pathogenesis of human diabetes mellitus Young suggested in 1939 (39) that a short period of spontaneous pituitary hyperactivity could induce a stimulation of the beta-cells which eventually would lead to exhaustion of the beta-cells and a clinical diabetes mellitus

There is however still not very much data in the current literature on which to estimate the significance of growth hormone in the pathogenesis of diabetes mellitus

Serum growth hormone can be determined by the classical tibia test method although the sensitivity of this procedure is quite low or by Berson and Yalow s radio-immunological technique In addition the growth hormone dependent Sulfation Factor (SF) can be determined by the method of Daughaday et al (10) This factor stimulates the uptake of radioactive sulfate in the costal cartilage of hypophysectomized rats It decreases after hypophysectomy and increases after injection of growth hormone (3 10 36)

The few publications dealing with the problem of serum growth hormone in diabetic patients have employed one or the other of these methods (12 13 19 32 34)

The following is a report of a study of serum SF in a large group of diabetics A comparative study of serum SF and immunological serum growth hormone as well as studies of immunological serum growth hormone during glucose loading will be published elsewhere (36 37)

MATERIAL

The series of patients studied was a random group of 60 newly-diagnosed, untreated diabetics admitted to the Second University Clinic of Internal Medicine Aarhus Kommunehospital. Twenty seven were obese and 33 non-obese. Obesity was defined as a weight $\geq 115\%$ of ideal

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Table I *Average values of serum SF±S E M in non obese controls and various types of diabetes mellitus*

	No of pats	Sulfation factor (mean±S E M)	Weight ( )
	33 diabetics	0 78±0 03	<115
	27 diabetics	0 80±0 02	≥115
A	60 diabetics	0 79±0 02	—
B	22 diabetics (proliferative retinopathy)	0 73±0 04	—
C	12 non diabetics	0 98±0 05	<115

A<C $p<0\,001$ B<C $p<0\,001$

weight based on Hafnia Life Insurance Table (30) Average male and female weights in the age group 30–34 years in this table served as ideal weight The table however refers to fully dressed individuals whereas the subjects included in the present material were weighed without clothes consequently "ideal weight was reduced by 1.5 kg and 1 0 kg for male and female subjects respectively

The material also in luded 22 long term diabetics with severe diabetic angiopathy These patients had not received insulin therapy for 24 hours before blood samples were taken

The control group consisted of 12 subjects of normal weight with a normal fasting blood sugar and without fasting glucosuria.

The age of the diabetic patients varied between 12 and years the range for the controls was 21–37 years of

SF and glucose were determined in serum obtained from fasting subjects In seven of the non-obese diabetics glucose loading (100 g glucose orally) was performed Serum glucose and serum SF were determined in the fastin state and one hour after glucose The degree of diabetes varied in the individual patients but all had a fasting blood sugar above 120 mg per 100 ml

METHODS

All blood samples were drawn from a cubital vein at 8–9 a m. after an overnight fast and rest, before the subject got out of bed. Immediately after venepuncture the blood was kept at +4 C for an hour and then spun at room temperature for 5 min at 3000 rpm in an ordinary laboratory centrifuge The serum was stored at −20 C until analysis was performed

Serum SF was d termined by a modification of the original SF method as described in an earlier paper (35) In this technique costal cartilage was used from *nonhypophysectomi ed* young rats after a two-day fast The index of precision obtained by using this technique is of the same order of magnitude as that described by Almqvist (2) employing Dau haday et al s method (10) with a few modifi ations One unit (U) of SF activity is defined as the activity of one ml of the pooled reference serum (35)

Serum glucose was determined by the glucoseoxidase method (18)

RESULTS

SF in serum from fasting subjects

Table I shows the average values in obese and non-obese newly diagnosed diabetics in patients with proliferative retinopathy as well as in ... non diabetics It appears that the average serum SF is considerably lower in the three diabetic groups than in the nondiabetics this difference is statistically highly significant Between the individual groups of diabetics however no difference could be demonstrated Nor could any s x difference be demonstrated in the different diabetic groups

The relationship between serum SF and serum glucose in newly diagnosed diabetics is shown in Fig 1 (non-obese) and Fig 2 (obese) As seen from Fig 1 a negative correlation between serum SF and serum glucose can be demonstrated for the non obese diabetics serum SF being lower at higher blood sugar levels The regression coefficient is −111 05 and the difference of the slope from zero is statistically significant ($p<0\,05$) Fig 2 shows that no such correlation is found in obese diabetics

Effect of administration of glucose on serum SF

Fig 3 shows the serum SF values in the fasting state and one hour after glucose loading in seven newly diagnosed non-obese untreated diabetics After glucose the serum SF fell in all cases although in some the fall was very pronounced and in others only slight The mean decrease of serum SF was 27 5 % which is statistically significant ($p<0\,05$)

DISCUSSION

The nature of the sulfation factor in serum is not known Physiological amounts of growth hormone added in vitro to the incubation mixture have no stimulatory effect on the sulfate uptake of isolated rat cartilage (2 28) The stability of the serum sulfation factor is not identical with that of immunologically detectable growth hormone Serum sulfation factor activity is stable for at least one week at room temperature (2 7) while the amount of immunodetectable plasma growth hormone declines when plasma is placed for more than 24 hours at room temperature (33) Serum sulfation factor is thus not identical with immuno-

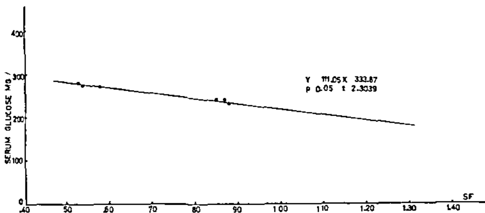


Fig 1 Relation between serum SF and serum glucose in 33 newly diagnosed untreated non-obese diabetics.

detectable growth hormone Daughaday et al (10) and Almqvist (1) have shown that the sulfation factor in serum from hypopituitary patients is low and that it increases after the patient has been treated with growth hormone injections It has also been shown that acromegalic patients have elevated values of SF (9 10 20 22) These results indicate that the serum sulfaction factor is growth hormone dependent

One of the results of the present study is the demonstration of a clear-cut reduction in serum SF in diabetic patients

It is known that various other substances in fluence the uptake of radioactive sulfate in costal cartilage Insulin in high concentrations (1–10 mU per ml) has been demonstrated to promote sulfate uptake (27 29) Insulin concentrations of this order of magnitude have not been described in normal subjects or diabetic patients and could thus not account for the differences obtained between our groups of diabetics and non-diabetics

Salmon and Daughaday (29) found that the omission of one of the following amino acids—valine threonine isoleucine or leucine—from the incubation medium resulted in a decreased sulfate uptake in costal cartilage Our incubation medium contains no amino acids except for those supplied by the serum to be assayed There is however no evidence of highly abnormal plasma concentrations of these aminoacids in diabetics Carlsen et al

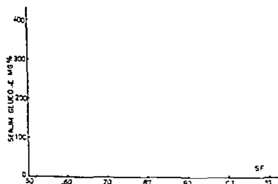


Fig Relation between serum SF and serum glucose in 7 newly diagnosed untreated obese diabetics

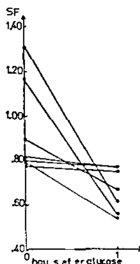


Fig 3 Serum SF levels in seven newly diagnosed untreated non-obese diabetics before and one hour after a glucose load (100 g)

(6) found only minor differences between diabetics and non diabetics in the serum concentration of amino acids In the diabetics the concentration of valine isoleucine and leucine was slightly higher while that of threonine was slightly lower than in the non-diabetic group

The glucose concentration of the medium during incubation does not influence sulfate uptake Salmon (27) found no difference in the uptake when the incubation medium contained 0 or 200 mg glucose per 100 ml

The age range of the diabetic patients and of the control group in the present series is not identical however Almqvist and Rune and Daughaday et al (4 10) found that serum SF was not influenced by age in adult subjects

A high serum sulfate level in the serum will of course tend to lower serum SF by isotopic dilution However there is no reason to suspect sulfate retention in our patients all of whom had a serum creatinine of less than 1 5 mg per 100 ml

Corticosteroids and e trogens are known to inhibit the sulfate uptake in costal cartilage (11 25) Plasma cortisol is known to be normal in uncomplicated diabetics (26 31) Plasma estrogen values are not available in diabetics but as an indirect measure of this Horstmann (14) and later ...ek (24) found a normal urinary excretion ... estrogenic substances in large groups of diabetics

As a whole it seems therefore that the low value of serum SF found in the diabetic patients included in the present study must be accepted as an expression of an abnormality which is characteristic of the diabetic state

There are only a few studies of serum SF in diabetic patients Almqvist et al (3) determined serum SF in two long term diabetics and found values of 1 19 and 0 64 Jensen et al (21) compared serum SF in obese and non obese newly diagnosed untreated diabetics As in the present study no difference was observed Their material did not include non-diabetics Chesley et al (7) found a low serum sulfation activity in six pregnant diabetics compared to ten pregnant non diabetics

It appears from the results obtained in the present study that there is a negative correlation between serum SF and serum glucose in a large group of untreated non-obese diabetics It was also shown that when glucose was given to that type of diabetic a fall in serum SF occurred in all cases It is interesting to note that no correlation obtains between fasting serum glucose and immunodetectable growth hormone (37) On the other hand the fall in serum SF one hour after glucose is paralleled by the fall in immunological serum growth hormone (5 19 37)

From the studies of the response of immunodetectable plasma growth hormone to glucose in obese diabetics (5) it is known that thes obese patients are either "normal responders" "poor responders" or non responders Thus the characteristic pattern of growth hormone response to a glucose load i e a fall followed by a rise 4-6 hours later may be either normal absent or less pronounced than in normals Our group of diabetics may well include all these three types It is possible that a weak correlation betwe n serum SF and serum glucose may be obscured by the heterogenity of the group examined

The low SF in fasting serum from diabetics cannot be explained today Although serum SF is obviously related to growth hormone production in the pituitary gland there is no correlation between serum SF and immunologically determinable serum growth hormone (36) and serum growth hormone determined in this way is not low in diabetes mellitus (37)

The only clinical condition in which serum SF is known to be reduced is in hypopituitarism however there is no evidence of reduced anterior pituitary function in diabetes mellitus

It appears from the results obtained in the present study that in non-obese patients the reduction of serum SF is most conspicuous in patients with a high fasting blood sugar Such a correlation was not demonstrable in the obese whose fasting blood sugar was on the whole somewhat lower as was to be expected It would be of interest to see if it were possible to normalize the fasting serum SF by a strict normalization of the blood sugar level for a period of time

It has been shown that serum SF falls after glucose One hour after ingestion of 100 g of glucose when the blood sugar is high the serum SF values were usually distinctly lower than before This finding is in accordance with the lower fasting serum SF values in patients with high fasting blood sugar but the course of the serum SF throughout the day and during the night is not known and a high blood sugar after glucose

may not be comparable to a high fasting blood sugar

It is interesting to note that serum SF is low after glucose at a time when immunological detectable growth hormone is known to be falling

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HETERO ANTIBODY AGAINST BILE CANALICULI IN PATIENTS WITH CHRONIC CLINICALLY ACTIVE HEPATITIS

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Abstract Antibody to the bile canaliculi of bovine liver was demonstrated in the serum from five patients with chronic clinically active hepatitis, in one out of ten patients with systemic lupus erythematosus and in one out of thirty one patients with positive antinuclear factor who did not suffer from systemic lupus erythematosus The antibody was not detected in the serum from five patients with acute hepatitis eleven patients with chole cystitis and thirty five donors The antibody was hetero-logous since it could not be demonstrated to bile canali culi in normal human liver tissue nor to bile canaliculi in the liver tissue from two patients with chronic clinically active hepatitis The antibody seems to be specifically directed against the bile canaliculi and it was always found concurrently with antinuclear factor against normal human liver tissue Furthermore in the five patients suffer ing from chronic clinically active hepatitis the bile canali culi antibody was found together with smooth muscle anti body and glomerulus antibody

In the serum of patients with liver cirrhosis active chronic hepatitis and so-called lupoid hepatitis antibodies to cell nuclei (7 8) cytoplasm (9 12) and smooth muscle (4 13) have been found In cases of lupoid hepatitis and active chronic hepa titis antibodies to renal glomeruli have been demonstrated (13) These antibodies are not spe cies specific but react with both animal and hu man tissue and furthermore the cytoplasmic anti body is not organ specific In patients with biliary cirrhosis virus hepatitis and post necrotic cirrhosis Paronetto (10) demonstrated antibodies to bil ductular cells in the hepatic tissue from patients with active post necrotic cirrhosis or biliary cir rhosis but not to normal hepatic tissue

The purpose of this report is to present the find ing of an organ specific h terologous antibody to bile canaliculi in bovine liver found in the serum of patients with chronic clinically active hepatitis possibly complicated by liver cirrhosis

METHOD

The indirect method of Coons was used for the demon stration of antibodies to antigens in tissue sections (1) Tissue frozen in liquid oxygen immediately after death or biopsy and stored at -20 C was sectioned at 6 μ in a cryostat The unfixed sections were air-dried and then incubated first with inactivated serum and secondly with fluorescein-conjugated anti human globulin for 30 min in a moist chamber at room temperature After each period of incubation the sections were washed for 3 periods of 5 min in isotonic phosphate buffered saline at pH 7.2 Finally the sections were covered with phosphate buffered glycerol and glass slides The reading was made in a Zeiss fluorescence microscope with an Osram HBO 00 W mercury lamp

All the sera were first tested against bovine liver All tests were made both with undiluted serum and serum diluted 1/10 Only if the sera showed a positive reaction at a dilution of 1/10 were the results considered to be positive

MATERIAL

Serum was taken from five patients with chronic clinically active hepatitis, possibly complicated by cirrhosis, five patients with acute hepatitis, two patients with systemic lupus erythematosus (SLE) with liver affection eight patients with SLE without liver affection eleven patients with cholecystitis and thirty five blood donors The five patients with chronic clinically active hepatitis had been ill for more than six months Their clinical and laboratory findings are shown in Table I In three patients, A H L M and A M the diagnosis was verified through biopsy Two patients, L M and A M had signs of cirrhosis One patient, I B was not available for biopsy In another patient O C biopsy could not be performed be cause of a constantly decreased prothrombin time In the patients with acute hepatitis the illness disappeared after less than two months

All the ten patients with SLE fulfilled seven of the following ten criteria for SLE, slightly modified after Jessar et al (3) 1 erythematous skin lesions 2 constitu tional symptoms of cachexia, fever and weight loss

Table I *Clinical and laboratory findings in five patients with chronic clinically active hepatitis*

Pat	Sex	Age	Ascites	Hepatomegalia	Serum bilirubin (mg/100 ml)	Thymol turbidity test	Serum albumin (g/100 ml)	Serum gamma globulin (g 100 ml)	Serum alkaline phosphatase	Prothrombin (of normal)	Serum glutamic oxalacetic transaminase (U/l)
I B	♀	46	–	–	2 3	0 71	2 9	4 6	16 9 KA	0	146
A H	♀	56	–	–	6 0	0 59	3 0	3 0	23 5 KA	68	177*
L M	o	13	–	+	4 4	0 87	2 5	6 5	121 U/l	7	04
A M	♀	66	–	–	3 5	0 72	3 5	3 0	109 U/l	54	700*
O C	♀	53	+	–	6 5	0 50	2 5	3 7	275 U/l	16	1 0

Normal 2–11 King-Armstrong units   * Normal 3–12 U/l   Normal 22–64 U/l

3 absence of sepsis 4 arthralgia 5 renal disease 6 suppression of blood forming elements with leucopenia anaemia and thrombocytopenia 7 hypertrophy of lymph nodes liver or spleen 8 endocarditis 9 serous membrane effusion—pericardial pleural and peritoneal and 10 greater incidence in females Two of the patients with SLE had findings compatible with liver affection both had a positive thymol turbidity test and eleva ed serum glutamic oxalacetic acid transaminase and alkaline phosphatase One of the latter had furthermore hepatomegalia decreased serum albumin and elevated serum gamma globulin All the ten patients with SLE had a positive antinuclear factor (ANF) against cell nuclei of bovine liver None of the five patients with chronic clinically active hepatitis fulfilled the criteria for SLE as stated above

Furthermore the study comprised thirty-one patients who did not suffer from SLE, but who had a positive ANF against cell nuclei of bovine liver found at routine examination The majority of these patients suffered from rheumatoid arthritis They were not examined for liver diseases

RESULTS

As shown in Table II antibody to bile canaliculi of bovine liver was detected in all the five patients with chronic clinically active hepatitis in one patient with SLE without liver affection and in one patient with a positive ANF who did not suffer from SLE This patient had rheumatoid arthritis A positive reaction is presented in Fig 1

Table II *Antibodies against bile canaliculi demonstrated by the indirect immunofluorescent technique*

Diagnosis	No	Bile canaliculi antibody
Chronic, clinically active hepatitis	5	5
Acute hepatitis	5	0
SLE with liver involvement	2	0
SLE without liver involvement	8	1
Cholecystitis	11	0
Blood donors	35	0
Diseases with positive ANF excl SLE	31	1
Total number	97	7



Fig 1 Immunofluorescent staining of bile canaliculi in fresh frozen bovine liver treated with inactivated serum from a patient with chronic clinically active hepatitis and fluorescein-conjugated anti human globulin Original magnification × 500

In most of the sera the positive reaction could only be demonstrated in the diluted specimen be… of a non specific fluorescence in the undilut… specimen None of the remaining sera contained factor reacting to bile canaliculi Fig 2 shows a negative reaction

All sera containing bile canaliculi antibody were then tested employing the same technique and in the same dilutions for the possible presence of bile canaliculi antibody to normal human liver tissue and liver removed by biopsy from two patients with chronic clinically active hepatitis (L. M and A M) for the presence of antibody to smooth muscle in the stomach of mice and blood vessels in normal human liver tissue for antibody to glomeruli in mouse kidney and cytoplasmic antibody to parietal cells in mouse stomach and to tubuli in mouse kidney

It appears from Table III that none of the sera which *were found to react with bile canaliculi of* bovine liver reacted with bile canaliculi in normal human liver or liver specimens from the two patients whose sera reacted with bile canaliculi of bovine liver All seven sera were also tested for the presence of ANF to the nuclei of bovine liver and normal human liver tissue Six of them contained ANF *to the nuclei in both bovine liver and* normal human liver tissue whereas the serum



Fig 2 No staining of bile canaliculi in bovine liver treated with inactivated normal, human serum and fluorescein-conjugated *anti human globulin Original* magnification × 500

Table III *Distribution of antibodies against bile canaliculi nuclei smooth muscles and renal glomeruli in serum from seven patients with heterologous bile canaliculi antibody*

Pat.	Diagnosis	Antigens: Bile canaliculi in Bovine liver	Normal human liver	Liver biopsy from L. M	Liver biopsy from A M	Nuclei in Bovine liver	Normal human liver	Smooth muscles in Mouse stomach	Artery of human liver	Glomeruli in Mouse kidney
I B	Chronic clinically active hepatitis	+	−	−	−	+	+	+	+	+
A H	Chronic clinically active hepatitis	+	−	−	−	+	+	+	+	+
L. M	Chronic clinically active hepatitis	+	−	−	−	+	+	+	+	+
A. M	Chronic clinically active hepatitis	+	−	−	−	+	+	+	+	+
O C	Chronic clinically active hepatitis	+	−	−	−	−	+	+	+	+
A B	SLE without liver involvement	+	−	−	−	+	+	+	+	+
A L.	Rheumatoid arthritis	+ / +	− / −	− / −		+ / +	+ / +	− / −	− / −	− / −
No		7	0	0	0	6	7	5	5	5

Positive only in undiluted serum.

Table I *Clinical and laboratory findings in five patients with chronic clinically active hepatitis*

Pat	Sex	Age	Ascites	Hepato megalia	Serum bilirubin (mg/100 ml)	Thymol turbidity test	Serum albumin (g/100 ml)	Serum gamma globulin (g 100 ml)	Serum alkaline phosphatase	Prothrombin (% of normal)	Serum glutamic oxalacetic transaminase (U/l)
I B	♀	46	–	–	2 3	0 71	2 9	4 6	16 9 KA	0	146*
A H	♀	56	–	–	6 0	0 59	3.0	3 0	23 5 KA	68	2[illegible]7
L. M	♂	13	–	+	4 4	0 87	2 5	6 5	121 U/l	7	[illegible]04
A M	♀	66	–	–	3 5	0 72	3 5	3 0	109 U/l	54	[illegible]00
O C	♀	53	+	–	6 5	0 50	2 5	3 7	275 U/l	16	1[illegible]0

Normal 2–11 King-Armstrong units * Normal 3–12 U/l Normal 22–64 U/l

3 absence of sepsis 4 arthralgia 5 renal disease 6 suppression of blood forming elements with leucopenia, anaemia and thrombocytopenia 7 hypertrophy of lymph nodes liver or spleen 8 endocarditis 9 serous membrane effusion—pericardial pleural and peritoneal and 10 greater inciden e in females Two of the patients with SLE had findings compatible with liver affection both had a positive thymol turbidity test and elevated serum glutamic oxalacetic acid transaminase and alkaline phosphatase One of the latter had furthermore hepatomegalia decreased serum albumin and elevated serum gamma globulin All the ten patients with SLE had a positive antinuclear factor (ANF) against cell nuclei of bovine liver None of the five patients with chronic clinically active hepatitis fulfilled the criteria for SLE as stated above

Furthermore the study comprised thirty-one patients who did not suffer from SLE, but who had a positive ANF against cell nuclei of bovine liver found at routine examination The majority of these patients suffered from rheumatoid arthritis They were not examined for liver diseases

RESULTS

As shown in Table II antibody to bile canaliculi of bovine liver was detected in all the five patients with chronic clinically active hepatitis in one patient with SLE without liver affection and in one patient with a positive ANF who did not suffer from SLE This patient had rheumatoid arthritis A positive reaction is presented in Fig 1

Table II *Antibodies against bile canaliculi demonstrated by the indirect immunofluorescent technique*

Diagnosis	No	Bile canaliculi antibody
Chronic clinically active hepatitis	5	5
Acute hepatitis	5	0
SLE with liver involvement	2	0
SLE without liver involvement	8	1
Cholecystitis	11	0
Blood donors	35	0
Diseases with positive ANF excl SLE	31	1
Total number	97	7



Fig 1 Immunofluorescent staining of bile canaliculi in fresh frozen bovine liver treated with inactivated serum from a patient with chronic, clinically active hepatitis and fluorescein-conjugated anti human globulin Original magnification × 500

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SIGNS OF URINARY TRACT INFECTION IN A HEALTH SURVEY OF FEMALE HOSPITAL EMPLOYEES

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Abst act A health survey has been made of urinary tract infection in 1346 female hospital employees Pyuria in sediment of freshly voided urine was noted in 20 % and found to be an unspecific symptom of urinary-tract in fection Significant bacteriuria was recorded in 2 5 and pyelonephritis as defined was diagnosed in 1 6%

Pyuria and bacteriuria were more common in divorced and widowed women than in the others Women who had borne children had a higher frequency of pyuria than nulliparae Pyelonephritis was diagnosed more frequently in women over 39 years old. A difference in the frequency of pyuria was noted between occupational groups and also between the personnel of a large somatic hospital and the personnel of a lar_e mental hospital.

This paper reports studies of the frequency of bacteriuria and other signs of urinary tract infec tion in female hospital personnel employed by Malmohus County Council In addition to studies of the frequency of pathological findings indicat ing urinary tract infection the investigation also included various factors such as age civil status parity occupation and place of work which might play a part in the pathogenesis

MATERIAL

In connection with a gynaecologi al health survey of County council hospital personnel at Lund the urine of 1346 women between 1? and 69 years old was examined by screening 696 of the n tig ted women were also a ked to complete a questionna re concern ng mainly ur nary-tract diseases. Because of pathological f ndings in the urine and or h stones sugge ti e of urinary tract disease 644 women were examined l n cally Among the screened women 843 worked in a la ge g ne al hospital (Lunds lasarett) 383 in a large mental hospital (St. Lars) and 73 n small institutions, su h as nurs ng homes homes for the aged, and childrens homes.

METHODS

A *Screening*

Urine specimens were collected before the gynaecological examination which was made between 7 and 8 o clock in the morning The women were instructed to void into sterile vessels after careful vulval cleansing with com presses soaked with sterile saline solution. The urine speci mens were then immediately cooled to about +4 C

All the specimens were examined by the following procedures

1 *Quantitative i ne cultu e* Some of the specimens were transferred to a sterile tube (cooled to about +4 C) and sent to the hospital s bacteriological laboratory (Head Prof R. Grubb) for semiquantitative urine culture. The bacterial count was quantitated according to the scale no growth less than 10^4 10^{4-5} and more than 10^5 bacteria per ml of urine

Sed ment Ten ml of well mixed urine were centrifuged for 5 min at 3000 rpm. The supernatant was decanted away so that 3 or 4 drops rema ned The sediment was shaken and 1 drop of urine was examined under the cover glass at magnif cation x 3?0

3 *Tests fo p ote ni ia* Albustix according to the maker s instruction and precipitation with sulphosalicylic acid (0%)

4 Uroscreen, the triphenyl tetrazolium-chloride (T T C) test (8) Hemastix and Clinistix

B *Cl n cal exam nat on*

The women whose urine showed any of the following abnormal ues were examined clinically mo e than 10^5 bacteria per ml of urine more than 5 white cells per high power f eld more than 5 red cells per high power field high bacterial counts in the sediment, Albustix read ngs of + + or higher pos t e sulphosalicyl c acid test gl co uria, positi e Uroscre n or T T C test, pos ti e Hem tix or Clin ti

635 (47 %) of the total number of 1346 women under went the cl nic l exam nation wh ch took place w th n 1 to 8 weeks after the screening All the aforementioned r nalyses were repeated at the cl nical exam nation and n add tion the 12-hour e cretion of formed el m nts in

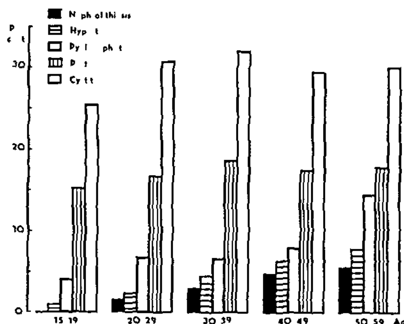


Fig 1 Frequency of nephrolithiasis, hypertension pyelonephritis proteinuria and cystitis in the history of the 696 women who completed the questionnaire at the screening examination

the specimens was calculated by Addis count and specific gravity of the urine was determined after 20 hours of water deprivation. The sampling technique was the same as that used at the screening but the specimens were usually collected later in the day The clinical examination included a careful history and a medical examination addition to the aforementioned urinalyses the following ratory procedures were also carried out determination Hb ESR antistreptolysin titre and serum creatinine

A radiological investigation, including plain X ray of the urinary tract and intravenous pyelography was made on special indications in 257 women

C *Definitions*

(*a*) *Bacteriuria* A bacterial count of more than 10^5 organisms per ml of urine in one specimen denoted *bacteriuria* a count of 10^{3-4} organisms per ml of urine denoted *suspected bacteriuria* a count of more than 10^5 organisms per ml of urine in two consecutive cultures with identical bacterial flora denoted *significant bacteriuria*

(*b*) *Pyuria* More than 5 WBC HPF or more than 4 mill WBC/12 h

(*c*) *Pyelonephritis* In accordance with Örsten (39) we considered that pyelonephritis was present when the case fulfilled at least thr e of the following criteria

1 A history suggestive of pyelonephritis
2 Significant bacteriuria
3 Impaired maximal concentration capacity (specific gravity $< 1\,025$ urine volume < 300 ml per 12 hours)
4 X ray findings interpreted as evidence of pyelonephritis or an anatomical change with an increased tendency to pyelonephritis (urinary tract malformations, nephrolithiasis, and hydronephrosis)
5 Pyuria (more than 4 mill. WBC/12 h)

(*d*) *Suspected pyelonephritis or urinary-tract infection* When two of the said criteria were present, the condition was classed as suspected pyelonephritis or urinary tract infection

D *Grouping of the investigated women*

In the analysis of the results the women were grouped by age civil status, parity occupation, and place of work. With respect to occupation, they were divided into three categories A B and C A = nurses and their equals B = auxiliary nursing personnel and their equals C = a mixed group comprising senior student nurses office staff and others.

RESULTS

A *A History of Renal Disease*

Fig 1 shows the frequency of previous urinary tract disease and high blood pressure according to the questionnaire

1 *Cystitis* Altogether 209 or 30% of the 696 women had had cystitis The frequency was independent of age civil status occupation and place of work

2 *Pyelonephritis* 53 women or 7 6% had had pyelonephritis The frequency was significantly higher for the women over 49 years old ($p < 0\,05$ (Fig 1)) With respect to civil status parity occupation and place of work there were no significant deviations

3 *Nephrolithiasis* 19 or 2 7% of the women said that they had had renal stones earlier

4 *Hypertension* 28 or 4 0% of the women

Table I *Frequency of urinary tract diseases in the history and urinary findings in the screened women in relation to age civil status parity occupation and place of work*

Screening examination	History: No of women examined	Cystitis ( )	Pyelonephritis ( )	Nephrolithiasis ( )	Hypertension ( )	Laboratory findings: No of women examined	Pyuria WBC/HPF ( ) >5	>10	Bacteriuria organisms ml ( ) ≥10⁴	>10⁵
Age (y)										
15–19	99	25 5	4 1	—	1 0	131	18 0	4 9	4 6	2 3
20– 9	255	30 8	6 8	1 6	2.4	460	18 6	10 0	11 7	4 6
30–39	123	32 1	6 7	3 0	4 5	260	19 5	10 4	8 4	5 8
40–49	126	29 6	8 0	4 8	6 4	253	0 9	10 9	9 6	5
50–69	90	30 3	14 6	5 6	7 9	237	21 0	11 7	12.6	5 6
Civil status										
Unmarried	325	27 4	5 8	2 2	0 9	555	17 7	7 0	7 4	3 1
Married	314	31 8	8 0	6 1	5 1	688	17 9	9 7	11 6	5 8
Divorced	31	35 5	19 4	6 5	6 5	45	24 4	15 6	17 8	13 3
Widowed	15	33 3	16 7	6 7	13 3	32	28 1	21 9	9 4	3 1
Parity										
0– para	353	30 0	6 5	2 8	2 0	705	17 6	7 1	8 9	3 5
1 para	121	31 4	9 9	4 1	5 8	234	17 5	11 1	9 8	4 3
2 para	129	34 9	7 8	7 0	3 9	231	18 1	9 1	9 5	6 1
≥3– para	90	23.3	8 9	5 6	4 4	172	23 3	14 5	14 5	8 7
Occupational category										
A	331	28 7	9 1	2.9	3 9	441	12.2	6 5	9 6	4 7
B	195	34 9	7 7	0 8	5 6	481	2 6	11 6	8 7	4 8
C	137	28 5	5 1	0 5	2.9	367	20 7	9 3	12 3	5 2
Place of work										
University hospital	175	30 9	8 0	7 4	7 4	843	20 0	10 3	9 4	5 0
St Lars hospital	381	28 1	8 1	3 7	1 8	383	12.8	7 0	10 7	4 7
Others	73	34 2	5 5	1 4	2.7	73	19 2	4 1	12 3	2 7
Total	696	30 3	7 6	2 7	4 0	1346	19 6	10 0	10 3	4 9

had a history of high blood pressure The frequency of renal stone and high blood pressure increased as a percentage but not significantly with age

B Laboratory Findings at the Screening

1 Pyuria defined as more than 5 WBC/HPF was noted in 244 (19 6%) of 1346 investigated women The results are summarized in Table I

Age civil status parity The frequency was largely the same in the different age groups As regards civil status there were no significant relations but the frequency of pyuria was higher as a percentage for divorced and widowed women than for the others (26 0 as against 17 8%) Nor was there any significant variation for parity but the percentage was higher for women who had borne three or more children than for the others (23 3 as against 17 7%) See Table I

Occupation The frequency of pyuria as a percentage was higher in categories B (auxiliary nursing personnel) and C (mixed group) than in category A (nurses) The difference was significant in the total material between categories B (22 6 %) and C (20 7%) on the one hand and category A (12 3 %) on the other ($p < 0\ 0005$ and $p < 0\ 005$ respectively) and in the age groups 20–29 40–49 and 50–59 between categories B and A ($p < 0\ 025$ $p < 0\ 01$ and $p < 0\ 025$ respectively)

Place of work The frequency of pyuria was higher in the general hospital than in the mental hospital in all age groups except the lowest (in total 20 0% as against 12 8) The difference was significant for the whole series and for the age groups 30–39 and 40–49 ($p < 0\ 005$ $p < 0\ 05$ and $p < 0\ 025$ respectively) In order to exclude the possibility that the difference in frequency between the places of work was due to different

distribution of occupational categories a comparison was made between the same categories of personnel at the two hospitals It was found that both in category A and in category B the frequency was higher in the general hospital for the age groups above 20 years The difference was significant for the age group 30–69 years ($p < 0.005$ and $p < 0.05$ respectively)

2 *Pyuria defined as more than 10 WBC/HPF* was noted in 125 (10 0°) of 1346 women whose urine was examined with respect to sediment

Age There was no significant variation with age but the frequency in the age group 15–19 years was lower than in the others (4 9% as against 10 0–11 7%)

Civil status The frequency was significantly higher for the divorced and widowed women than for the rest in the total series ($p < 0.01$) There was no significant difference between married and unmarried women

Parity The frequency was throughout lower for nulliparae than for women who had borne children and the difference was significant for the total series (7 1% as against 11 3 % $p < 0.01$)

Occupation As regards the frequency in different occupational categories the result was the same as that for pyuria defined as more than 5 white cells per high power field that is a higher frequency for most of the age groups in categories B and C than in category A The figures in the respective age groups were too low for statistical treatment but in the total series there was a significant preponderance in category B in comparison with category A ($p < 0.01$)

Place of work Similarly to the result for pyuria defined as more than 5 white cells per high power field the frequency was higher in all the age groups in the general hospital but the difference was not significant When a comparison was made between the occupational categories the result was still the same the difference being significant for category A in the age group 30–69 ($p < 0.05$)

3 *Quantitative urine culture*

(*a*) *10³ or more bacteria per ml of urine* 135 (10 3%) of 1346 women had 10³ or more bacteria per ml of urine

Age The frequency was lower in the women under 20 years old than in the higher ages ($p < 0.05$) No other variation with age was noted

Civil status The frequency was significantly higher both for married and for divorced women than for unmarried women ($p < 0.025$ and $p < 0.05$ respectively)

Parity The frequency was significantly higher for women who had borne three or more children than for the nulliparae (14 5 as against 8 9° $p < 0.05$) No other distinct tendencies were noted

Occupation Significant differences between the occupational groups were noted only in the age group 20–29 years in which the frequency was significantly higher in category C than in categories B and A (19 7% as against 8 8 and 10 1% respectively $p < 0.05$ in both cases) and in the age group 30–39 years in which the frequency was higher for auxiliary nursing personnel than for nurses (16 2% as against 5 4% $p < 0.05$)

Place of work There was no correlation between frequency and place of work

(*b*) *More than 10 bacteria per ml of urine* 66 (4 9%) of 1346 women had more than 10 bacteria per ml of urine

Age Similarly to the results under (*a*) the frequency was lower in the lowest age group than in the others (2 3 as against 4 6–5 8) the difference was not significant

Civil status The frequency was significantly higher for married and for divorced women than for unmarried women (5 8% and 13 3% respectively as against 3 1% $p < 0.05$ and $p < 0.005$ respectively)

Parity As regards parity a tendency similar to that for women with 10³ or more bacteria per ml of urine was noted that is a higher frequency for women who had borne two or more children than for nulliparae (7 2 % as against 3 5 $p < 0.025$)

Occupation place of work There was no noteworthy difference between the occupational groups or between places of work

C Clinical Examination

635 out of the 1346 women who underwent the screening tests attended the clinical examination This group did not differ significantly from the primary material with respect to age civil status parity occupation or place of work

1 *Pyelonephritis*

Pyelonephritis according to the definition used in this study was diagnosed in 21 (3 3%) of the 635

Table II *Frequency of pyuria bacteriuria suspected pyelo nephritis/urinary tract infection and pyelonephritis in the 635 clinically examined women in relation to age civil status parity occupation and place of work*

	No of women examined	Addis count WBC/12 h >4 mill ( )	Addis count WBC/12 h >10 mill ( )	Quantitative urine culture $>10^5$ bact /ml ( )	Quantitative urine culture Significant bacteriuria ( )	Suspected pyelo nephritis ( )	Pyelo nephritis ( )
Age							
15–19	55	36 4	14 5	3 6	3 6	5 5	1 8
0– 9	245	34 7	15 5	4 5	2 4	4 5	0 8
30–39	115	27 8	13 9	10 4	9 6	16 5	1 7
40–49	114	33 3	19 3	6 1	6 1	7 9	4 4
50–69	106	35 8	25 5	8 5	4 7	19 8	9 4
Civil status							
Unmarried	266	33 5	15 8	5 6	3 4	7 5	1 8
Married	325	32 3	16 9	6 8	5 8	11 1	3 7
Divorced	25	32 0	32 0	8 0	8 0	.0 0	12 0
W dowed	16	56 0	37 5	12 5	6 3	6 3	12 5
Parity							
0– para	321	32 2	15 8	5 3	3 7	7 4	2 2
1– para	109	36 7	17 6	3 7	8	9 2	5 5
2 para	117	33 3	17 9	8 5	5 1	10 3	1 7
≥3 para	86	34 9	19 8	11 6	11 6	18 6	7 0
O cupational category							
A	193	28 5	16 1	6 2	5 7	8 3	2 9
B	224	40 6	23 7	7 1	4 5	10 3	4 5
C	167	39 5	15 6	7 8	6 9	13 8	2 2
Place of work							
University hospital	433	34 6	18 .	6 5	4 6	9 2	3 0
St Lars hospital	170	27 0	14 1	5 9	5 3	11 8	4 1
Others	25	48 0	28 0	12 0	8 0	2 1	4 0
Total	635	33 4	17 5	6 5	4 9	9 9	3 3

women examined clinically (unless otherwise stated the percentages reported in the following are cal culated on the number of clinically examined women) This means that pyelonephritis was diag nosed in 1 6 % of the 1346 women who took part in the health survey

As regards the criteria used for the diagnosis of pyelonephritis pyuria was present in all cases Next in order was a history of pyelonephritis (14 cases) followed by radiographic changes (13 cases) Significant bacteriuria was present in seven women that is one third of the cases and im paired concentration capacity in six women

Age It will be seen from Table II that the frequency of pyelonephritis was clearly higher in women over 39 years old than in younger women (7 3% as against 1 2 $p<0\,0005$)

Civil status The frequency was higher for di vorced and widowed women than for the rest and higher for married than for unmarried women

The figures were 12 2 for divorced and wid owed 3 7 for married and 1 8° for unmarried women The figures were too low for statistical treatment

Parity The frequency was higher for women who had borne three or more children than for women who had borne fewer or no children (7 0° as against 2 7 the figures were too small for statistical treatment)

Occupation The frequency was higher for cate gory B than for categories A and C (4 5 as against 2 9 and 2 2 respectively the figures were too low for statistical treatment)

Place of work There was no noteworthy differ ence in frequency between places of work

At the screening examination of the 21 women in whom pyelonephritis was diagnosed eight stated that they had had proteinuria Two women had had cystitis one had had hypertension and three had had renal stones

The screening analysis of the urine showed Albustix++ or more in two cases positive sulpho salicylic acid tests in six more than 5 WBC/HPF in eleven and 10^3 or more bacteria per ml of urine also in eleven cases

2 *Suspected pyelonephritis or urinary tract infection* 62 (9 9 %) of the 635 clinically examined women fulfilled two of the five afore mentioned criteria for a diagnosis of pyelonephritis These cases comprised 4 7 % of the total series

The most common finding was pyuria which was present in 48 (77 %) of the 62 women Second in order were significant bacteriuria and radio graphic changes each present in 21 women (34 %) Impaired concentration capacity was noted in 10 cases (16 %) and 24 cases (39 %) had a history of pyelonephritis

Age The percentage of women who fulfilled two criteria for pyelonephritis varied significantly with age ($p < 0\,0005$) A significantly lower frequency was recorded for women under 30 years of age (4 5–5 5 %) than for the higher age groups (7 9–19 8 %) The frequency in the age group 40–49 years was significantly lower than in the age group 50–69 years (7 9 as against 19 8 % $p < 0\,025$) and lower as a percentage but not significantly in the age group 30–39 years (16 5 %)

Civil status occupation place of work The number of cases did not permit statistical calculation but as a percentage there was no distinct tendency with respect to the distribution by civil status occupational groups or places of work

Parity The frequency of suspected pyelonephritis increased as a percentage with increasing number of childbirths (from 7 4 % for nulliparae to 18 6 % in women who had borne three or more children) The frequency for women who had borne two or more children was significantly higher than for those who had borne one or no child ($p < 0\,01$)

Seventeen (28 %) of the 62 women with urinary tract infection or suspected pyelonephritis stated that they had had proteinuria and nine (15 %) had had cystitis Six (10 %) stated that they had had hypertension and six (10 %) had had renal stones The urinalysis at the screening examination showed Albustix++ or more in four (6 %) positive sulphosalicylic acid test in seven (11 %) more than 5 WBC/HPF in 32 (51 %) and 10^3 or more bacteria per ml of urine in 29 cases (46 %)

If the above groups under 1 and 2 are combined it will be found that 84 out of the 1346 investigated women (or 6 3 %) showed symptoms which according to our criteria indicated urinary tract infection or suspected or probable pyelonephritis

In the combined group of women who fulfilled two or more criteria for pyelonephritis there was the same relation to age civil status parity occupation and place of work as in the separate groups but in addition a significant difference was noted between auxiliary nursing personnel and nurses in the age group 20–39 years (8 4 % as against 2 4 % $p < 0\,01$)

3 *Quantitative sediment*

(a) *More than 4 mill WBC/12 h* (without a history or other evidence of urinary tract infection) Out of the 635 clinically examined women 213 (33 5 %) had pyuria alone

Age civil status parity The frequency of pyuria showed no noteworthy relations to age parity or civil status

Occupation The frequency of pyuria in the age groups above 19 years was higher in categories B and C than in category A The difference between B and A in the age group 40–69 years was significant (44 8 % as against 22 2 % $p < 0\,005$)

Place of work In the age groups above 19 years the frequency of pyuria was higher in the general hospital than in the mental hospital the difference being significant in the age group 50–69 years (48 % as against 14 3 % $p < 0\,005$) The possibility cannot be excluded however that this difference was due to an overrepresentation of auxiliary nursing personnel among the examined women from the general hospital

(b) *More than 10 mill WBC/12 h* 111 (17 5 %) of the 635 examined women had more than 10 mill white cells per 12 h without history or other evidence of urinary tract infection

Age There was no significant variation with age but the frequency as a percentage was higher in the age group 50–69 years than in the other age groups (25 5 % as against 13 9–19 3 %)

Civil status The frequency was significantly higher for divorced and widowed women than for married and for unmarried women (34 1 % as against 16 9 % and 15 8 % respectively $p < 0\,025$ and $p < 0\,01$ respectively)

Parity No distinct tendency was noted with respect to the number of pregnancies and the frequency of pyuria

Occupation In the age groups above 19 years the frequency was higher in category B than in categories A and C between which there was no difference The difference was significant in the combined age group 20–69 years (for category B 25 7 for A 15 7% and for C 16 0% $p <$ 0 025 and $p <$ 0 05 respectively)

Place of work In the age groups above 19 years the frequency was higher in the general hospital than in the mental hospital The differences were not significant and could possibly also be due to a dissimilarity in the number of examined women in the respective occupational groups from the two hospitals

DISCUSSION

General Comments

The terms pyuria bacteriuria urinary tract infection and pyelonephritis have not been uniformly defined in the literature With a technique of analysis similar to that used by us at the screening examination the borderline for pyuria ranged from 3 to 20 white cells per high power field (31) As regards leucocyturia in quantitative sediment there are also differences of opinion about the normal range (3 19 27)

As to the clinical significance it should be emphasized that pyuria as the only symptom need not be equivalent to urinary tract infection and that such infection can often occur without pyuria This holds true for the assessment of single tests in particular The relation between pyuria and bacteriuria varies from one published report to another (33)

In accordance with Kass (8 9 10 11) definitions of significant bacteriuria the borderline between contamination and urinary tract infection has generally been set at 10 organisms per ml in two consecutive cultures with identical bacterial flora although opinions differ on this point as well (14)

The potential part played by significant bacteriuria in the development of pyelonephritis has been convincingly verified by several authors (6 9 10 11 13 14 20 22 37)

To overcome the difficulty of classifying different types of infection of the urinary tract, Freedman (5) simply lumped all of them together under the term urinary tract infection

Some uncertainty attaches to the definition of pyelonephritis chosen by us (according to Örsten (39)) and overdiagnosis as well as underdiagnosis will undoubtedly occur in our series

Because of the varying clinical definitions of pyelonephritis it is difficult to compare different published materials At the Second International Symposium on Pyelonephritis Strauss (30) therefore advised every author who writes about urinary tract infections to define the term he uses

Comments on our Results

The material presented must be regarded as selected since it comprises hospital personnel In Sweden a certificate of health is required for employment in hospitals and this may mean that some persons with pyelonephritis without subjective symptoms will be rejected On the other hand the possibility cannot be excluded that with the bacterially contaminated hospital environment the risk of infections of the urinary tract is also greater

History The assessment of historical data such as those relating to cystitis mostly diagnosed by the patients themselves is bound to be very uncertain Psychogenic micturition disturbances and uncharacteristic dysuria are often intepreted by the patient as cystitis On the other hand it is to be expected that the usually mild disorders caused by cystitis will soon be forgotten and this might explain the relatively small proportion (30) of women with a history of cystitis in our series For the sake of comparison it may be mentioned that Sallmander and Öst (26) in a control series of non bacteriuric women found that 50% of them had a history of urinary tract infection The figures for the frequency of pyelonephritis (7 6) of renal stone (2 7 %) and of high blood pressure (4 0%) seem to be more reliable since these diagnoses are usually based on data supplied by a physician

Laboratory procedures Published figures for the frequency of *pyuria* in non bacteriuric women show great variation probably owing to the aforementioned factors but the frequency in our series 19 6 is fairly close to the reported mean figure of 17 8% which one of us (33) found in a survey of the relevant literature

Data on the frequency of asymptomatic *bacteriuria* also vary (6 8 19 23 35) Our figures for the frequency of bacteriuria (more than 10 organisms per ml of urine in one culture 4 9 % in two cultures 2 5 %) agree well with the frequency reported by Kass (10) and by Sallmander and Öst (26) for subjectively healthy women namely 4 4 % and 5 0 % respectively Our low figure for significant bacteriuria based on two consecutive cultures might be explained by the long interval between the cultures and by the fact that the second urine specimen represented day urine with short bladder incubation

A diagnosis of pyelonephritis according to our criteria was established in only 21 (1 6 %) of the 1346 women taking part in the health survey If the women who had suspected pyelonephritis or urinary tract infection are added to the pyelonephritis group the frequency will be 6 3 %

The frequency of pyelonephritis as well as that of suspected pyelonephritis or urinary tract infection was significantly higher in the higher age groups over 29 years which is in agreement with general experience

Most authors (9 11 17 23 26) have found that prevalence of asymptomatic bacteriuria in ...es with age but Vejlsgaard (35) noted a per... increase only after the age of 70 in his series As regards bacteriuria in pregnancy there is a difference of opinion about its relation to age Some authors (6 7 11 25 32 37) found that the frequency rose with increasing age whereas others noted no age difference (2 38) or the highest frequency at the age of 20 (11 9 % as against 8 3 % at the age of 30 (16)) The age variation of asymptomatic bacteriuria has been attributed to various factors such as sexual activity pregnancies gynaecological disorders and social conditions In a series of patients with symptomatic urinary tract infections Loudon and Greenshalgh (18) noted a peak in the age group 20–29 years then there was a fall followed by a second rise after the age of 70

In the present series the frequency of cystitis in the history did not vary with age On the other hand we noted as was expected that the frequency of pyuria showed no significant relation to age

The higher frequency of bacteriuria in the women over 19 years old in our series could primarily be presumed to be due to the fact that a greater number of women older than 19 years are married and have regular sexual intercourse It is also possible that completed pregnancies would play a part

As far as women are concerned marriage usually means changes in sexual habits and socio-economic status In addition the number of pregnancies is higher in married than in unmarried women Therefore higher prevalence of urinary tract infections in the married would not be unexpected and has in fact been reported by several authors Loudon and Greenshalgh (18) in their series of women with urinary tract infections found a higher frequency among married than among unmarried nulliparae Sleigh et al (29) examined 397 women who visited an infertility clinic and found a frequency of bacteriuria of 8 % whereas no case of bacteriuria was detected among 100 pupil midwives The higher frequency among the infertile women may possibly be explained in part by the instrumental interventions they had undergone because of their infertility Chalmers (2) found a frequency of bacteriuria of 5–6 % in married nulliparae as against 1 % in young unmarried nulliparae The difference was presumed to be due to different sexual activity

In our series the frequency of pyuria was higher in divorced and widowed women than in the rest both at the screening examination (more than 10 WBC/HPF) and at the clinical examination (more than 10 mill WBC/12 h) On the other hand there was no difference between unmarried and married women The frequency of bacteriuria was significantly higher in divorced and widowed and in married women than in unmarried women (9 1 % 5 8 % and 3 1 % respectively) The same tendency was noted for pyelonephritis although the difference was not significant (12 2 3 7 % and 1 8 % respectively)

The high frequency of latent and manifest urinary tract infection during pregnancy has in recent years attracted increased interest and many comprehensive investigations have been made into the subject The high prevalence has been attributed to the changes morphological and others which accompany pregnancy but it has also been suggested that increased sexual activity at the beginning of pregnancy would play a part It would therefore be reasonable to presume that the prevalence of bacteriuria would increase with the number of completed pregnancies as it has in fact been

found to do in some studies of pregnant (2 22 25 32 34 37) and non pregnant women (21) Vejlsgaard (35) and Williams et al (38) on the other hand noted no relation between bacteriuria and parity Kass (9) found a more distinctly increased frequency only after seven pregnancies Unlike these authors Forkman (4) and Petersdorff (24) noted a higher frequency in primigravidae than in multigravidae and Petersdorff concluded that there does not seem to be an increased rate of acquisition with parity

In our series there was a higher frequency of findings indicating urinary tract infection in women who had borne children than in nulliparae which would support the view that completed pregnancies would increase the prevalence of urinary tract infection The possibility cannot be excluded however that other factors such as sexual habits and social and hygienic conditions would play a more important part than pregnancy in itself

The fact that social and economic conditions play a part in the causation of certain diseases has long been recognized. In spite of the socioeconomic equalization that characterizes the development in many countries in the 20th century the social factors must still be considered to be of aetiological significance As regards urinary tract infection for instance the frequency has been found to vary between social groups living under different conditions Kass (11) noted a higher frequency of bacteriuria in the agricultural population than in the urban population in Jamaica and Turck et al (34) in accordance with Whalley (36) Beard and Roberts (1) recorded a higher frequency in pregnant women of lower socioeconomic status In this connection a parallel may be drawn to the observation that cervical cancer is more common in some socioeconomic groups than in others Since our investigation was planned mainly as a purely somatic and not as a sociological study we were not able to group the women according to social and economic class We therefore chose to make a rough division by occupation well aware of the fact that in Sweden a particular occupation has often no special relation to social and economic conditions The investigation showed that pyuria was throughout more common among the auxiliary nursing personnel than among nurses and that there was no such difference for bacteriuria It is possible that the higher frequency of pyuria among the auxiliary nursing personnel could be explained by unsatisfactory collection of urine specimens owing to less medical experience Another possibility which cannot be excluded is that the close contact that the women in this group have with the patients in their daily work might play a part

The accumulation of infections in a general hospital and the modern intensive antibacterial treatment which has led to the development of pathogenic nosocomial bacterial strains should mean that the personnel in such a hospital are exposed to greater risk of infections An interesting observation was made by Kennedy et al (12) who found that some serotypes of E coli which produce diseases of the urinary tract were more common in the intestines of hospital inpatients than in outpatients We therefore found it justifiable to compare the frequency of urinary tract infection among women working in a large general hospital with that in the personnel of a large mental hospital and small nursing institutions respectively The comparison showed that pyuria was more common in the general hospital personnel than in the others also when the composition of the personnel in the different hospitals was taken into consideration and that there was no difference in frequency as regards bacteriuria In view of the fact that pyuria as was mentioned in the foregoing is an unspecific sign of urinary tract infection the results do not justify the conclusion that the prevalence of urinary tract infections would be higher among the general hospital personnel

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ARE PROTEINURIA TESTS RELIABLE AS SCREENING METHODS FOR RENAL DISEASE?

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Abstract To assess the value of proteinuria tests as screening method for the detection of urinary tract disease an evaluation has been made of the Albustix and the sulphosalicylic acid tests in a health survey of 1346 women between 15 and 69 years old, all of whom are employed in hospitals.

The study comprised screening of the total material (1346 women) and a clinical examination of 634 women with pathological urine findings at the screening and/or histories suggestive of urinary tract disease The clinically examined women were tested with Albustix on four occasions and by the sulphosalicylic acid test on three occasions. At the screening 3.5% were Albustix positive and 5.9% positive in the sulphosalicylic acid test. In 33.1% there was a discrepancy between the two tests. Of the clinically examined women 0.3% were Albustix positive on all four occasions, 2.4% on two or three and 47.2% on one occasion. The corresponding figures for women examined by the sulphosalicylic acid test on three occasions were 1.7% positive on all three occasions, 6.8% positive on two and 22.3% on one occasion. The frequency of intermittently positive proteinuria tests decreased significantly with increasing age The frequency of urinary tract disease and hypertension was the same in the group with intermittently positive proteinuria tests as in the group with negative tests throughout. The results show that proteinuria tests are not suitable as the only screening method for the detection of urinary tract diseases

In our clinical work we have often observed discrepancies between the results of proteinuria tests and other clinical findings We have therefore undertaken to investigate the constancy of the Albustix results when the test was repeated on various occasions on the same subject during a health survey in women as well as its correlation to the sulphosalicylic acid test and other signs of renal disease Possible influences of various parameters—age marital status parity occupation and place of work—were also studied

In another study the same material was analysed for the frequency of symptoms indicative of urinary tract infection (15)

MATERIAL AND METHODS

The material comprised 1346 women whose ages ranged between 15 and 69 years and who were employed at the County Council hospitals in Lund The investigation, which was made in connection with a health survey included screening and clinical examination. Among the screened women 843 were working at a large general hospital (Lunds lasarett) 383 at a large mental hospital (St. Lars Sjukhus) and 73 at smaller institutions, such as nursing homes, homes for the aged, and children s homes.

The screening tests were made on fresh morning urine voided after careful vulval cleansing with sterile isotonic saline solution and comprised the following analyses

1 *Albustix* Urinary protein concentration estimated according to the manufacturer s grading. 2+ to 4+ were considered as positive reactions and 1+ as intermediate

2 Precipitation with 20% *sulphosalicylic acid* Weak precipitation was assessed as intermediate (+) and distinct to marked precipitation as positive +

3 Microscopy of *sediment* Pyuria was defined as more than 5 white cells per high power field and haematuria as more than 5 red cells per high power field.

The following methods were also applied quantitative urine culture the triphenyl tetrazolium-chloride (T T C) Clinistix, and Hemastix tests.

For technical reasons, only the later part (696 women) of the investigated material, which did not differ significantly in any way from the total material, were asked to complete a short questionnaire about renal and urinary tract diseases.

Those who had histories suggestive of renal disease or whose urines were abnormal in the screening tests were advised to appear for a clinical examination within 1 to 8 weeks. The 634 women who returned for this examination did not differ significantly from the screened women with respect to age marital status, parity occupation, and place of work

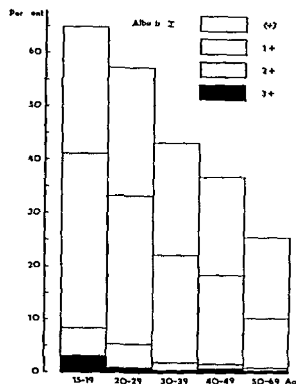


Fig 1 Albustix readings in different age-groups at the screening examination

The urinalyses included in the screening tests were repeated at the clinical examination. The Albustix test was used on three and the sulphosalicylic acid test on two occasions during the same 24 hour period (on submitted specimen of night urine for Addis count on freshly passed morning urine and, only for Albustix, on urine voided later in the day) When considered indicated a more comprehensive investigation was made including intravenous urography

Significant haematuria was recorded as present when *both the screening and the clinical examination showed* positive Hemastix readings

The women were grouped by occupation into hospital nurses (441) auxiliary nursing personnel (481) and a mixed group (367) comprising senior student nurses and office staff and others.

RESULTS

A *A History of Proteinuria and Hypertension*

Proteinuria 120 (17 2%) of 696 women had had proteinuria earlier The frequency was significantly higher in married than in unmarried women (20 7% and 13 5% respectively $p < 0\ 025$) For those formerly married (divorced plus widowed) the frequency was 19 6% A history of proteinuria was also more common among women who had borne children than among nulliparae (20 3% and 14 2% respectively $p < 0\ 05$) There was no significant difference between married and unmarried women without children (12 7% and 16 5% respectively) The percentag of women with a history of proteinuria was higher among those working at the big hospital than among those in the small institutions (18 8% and 6 8% respectively $p < 0\ 025$) There was no correlation between a history of proteinuria and occupation or age

High blood pressure 28 women (4 0%) had a history of high blood pressure The frequency had a tendency to increase with age though not significantly

B *Laboratory Findings at the Screening*

1 *Albustix*

Frequency of positive results The urines of 47 (3 5%) of the 1346 women read 2+ to 4+ and those of 292 (21 7%) 1+

Age (Fig 1) The frequency of Albustix (+) to 2+ fell gradually and significantly with increasing age ($p < 0\ 0005$)

Marital status parity occupation place of work There were small percentage differences but without any special tendency and not significant, in the frequency of proteinuria in relation to each of these parameters

Albustix–pyuria Table 1 shows the Albustix results in relation to the number of white cells per high power field in the sediment It will be seen that the frequency of pyuria defined as more than 5 white cells per field was clearly higher in women with intermediate or positive Albustix readings than in those with negative readings (31 3% as against 15 8% $p < 0\ 0005$) The frequency of pyuria was not significantly correlated with age

Table I *The results of the Albustix and the sulphosalicylic-acid tests at the screening examination compared with the frequency of pyuria on the same occasion*

Results	Albustix		Sulphosalicylic acid test	
	Number examined	Sediment > 5 WBC HPF (%)	Number examined	Sediment > 5 WBC HPF (%)
Negative	953	15 8	710	13 8
Intermediate	259	30 5	183	9
Positive	41	36 6	53	37 7

2 Sulphosalicylic acid test

Frequency Out of a total of 978 investigated women 59 (5 9 %) were positive and 187 (19 1 %) doubtfully positive

Age (Fig 2) The percentage of positive sulphosalicylic acid tests showed no significant correlation with age The percentage of doubtfully positive tests fell significantly with increasing age ($p < 0.0005$)

Marital status parity occupation place of work There were no noteworthy relations between a positive sulphosalicylic acid test and each of these parameters

Sulphosalicylic acid test–pyuria (Table I) Similarly to the Albustix results the frequency of pyuria was significantly higher in the women with doubtfully or clearly positive readings than in those with negative readings (30 1 % as against 13 8 %, $p < 0.0005$)

3 Albustix–sulphosalicylic acid test

(Table II) It will be seen from the table that a total number of 978 women were examined by both the Albustix and the sulphosalicylic acid test In 654 cases (66 9 %) the results of the two tests were in agreement (580 59 3 % negative 58 5 9 % doubtfully positive and 16 1 6 % positive) while in 324 (33 1 %) there was a discrepancy between the two tests Neither urinary pH nor pyuria could explain this discrepancy

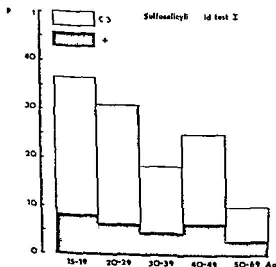


Fig Results of the sulphosalicylic acid test in different age groups at the screening examination

Table II *A comparison between the Albustix readings and the results of the sulphosalicylic acid test at the screening examination*

Albustix readings	Number examined	Sulphosalicylic acid test: Negative (%)	Intermediate (%)	Positive (%)
Negative 0; (+)	703	82 5	15 5	2 0
Intermediate +	231	62 8	25 1	12 1
Positive ≥2+	44	18 2	45 5	36 4

C Clinical Examination

1 Albustix

Frequency of positive Albustix (Table III) Forty six (7 3 %) of the 634 women who were clinically examined were positive (2+ to 4+) and 202 (31 9 %) were doubtfully positive (1+) at the screening examination

In Albustix tests repeated on three occasions at the clinical examination 187 (48 4 %) of the 386 women who were negative at the screening examination were negative throughout Two (0 5 %) were positive on all three occasions two (0 5 %) were positive on two occasions and 35 (9 0 %) *were positive on one occasion The rest (160)* were doubtfully positive on one or more occasions

The 202 women who were doubtfully positive in the screening test were negative in all the repeated tests except 24 (11 9 %) who were positive on one occasion

Of the 46 women who were positive at the screening examination 2 remained positive throughout 1 positive in two repeat tests and 10 *positive in one repeat test In the remaining 33* (71 7 %) the first positive screening results could not be reproduced

Accordingly of the 634 women who attended the clinical examination 2 (0 3 %) had positive readings on all four occasions including the screening test and 3 (0 5 %) in three tests out of four In 12 (1 9 %) the Albustix readings were positive twice and in 299 (47 2 %) once

History of proteinuria 147 (23 2 %) of the clinically examined women had had proteinuria earlier The proportion of those who had had proteinuria was significantly greater among those

Table III *Results of the three Albustix tests at the clinical examination in the groups with negative intermediate and positive readings at the screening examination*

0 = negative (+) = intermediate + = positive

Albustix screening	Number examined	Albustix readings in three tests at the clinical examination									
		000 ( )	00(+) ( )	0(+)(+) ( )	00+ ( )	0++ ( )	0(+)+ ( )	(+)(+)(+) ( )	(+)(+)+ ( )	(+)++ ( )	+++ ( )
Negative	386	48 3	39 3	2 3	7 5	0 5	1 6	—	—	—	0 5
Intermediate	202	32 3	51 8	4 0	9 5	—	2 5	0 5	—	—	—
Positive	46	26 1	43 5	2 2	17 4	2 2	4 3	—	—	—	4 3

with positive Albustix readings on one or more occasions than among the rest (52 9% as against 22 4% $p<0\ 01$)

Age It will be seen from Fig 3 that the proportion of positive Albustix readings in one test decreased significantly with age ($p<0\ 0005$) and that the proportion of positive readings in more than one test showed no special variation with age The two women who were positive throughout are found in the age group 40–49 years

Marital status The percentage of women in the age group 30–39 with one positive Albustix reading was significantly higher among the married than among the unmarried women (32 4 as against 2 5% $p<0\ 0005$) A tendency in the opposite direction though not significant was noted in the age group 20–29 (16 2 as against 25 0%)

Parity In the age groups 20–39 the percentage of women with one positive Albustix reading was higher for those who had borne children than for the nulliparae (26 0% as against 19 5% and 11 4% as against 8 0% for the age groups 20–29 and 30–39 respectively) The differences were not significant

Occupation No uniform tendency in the results was noted

Place of work The proportion of women with one positive Albustix reading in the age group 20–29 was significantly higher for the mental hospital than for the somatic hospital group (36 1% as against 15 1% $p<0\ 005$) The rest of the figures were too low for statistical analysis but no special tendency was noted

Blood pressure In the age group 30–39 the mean diastolic pressure was significantly lower for the women with one positive Albustix reading than for those with negative readings throughout (70 0 *mm* Hg as against 75 6 *mm* Hg $p<0\ 05$) The mean systolic pressure did not differ between the same categories of women Thirty two (5 0%) of the 634 investigated women had raised systolic pressure and 9 (1 4%) had raised diastolic pressure One of the 34 women with hypertension was positive throughout and three were positive on one or two occasions

Body weight height and weight/height No significant differences were noted in any of these parameters between the women with one positive Albustix reading and those who were negative throughout

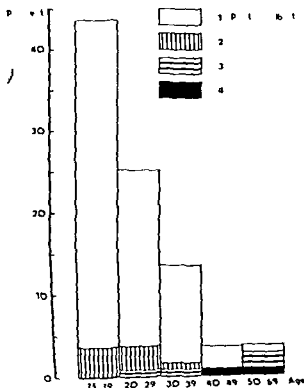


Fig 3 Frequency of positive Albustix readings on 1 2 3 and 4 occasions in 634 women

Table IV *Frequency of the different clinical diagnoses in relation to the results of the four Albustix tests*

Diagnosis	Albustix results 0000	000+	00++	0+++	++++	Total
Status post glomerulonephritidem	6	1	1	—	—	8
Hypertension	30	[illegible]	1	—	1	34
Chronic pyelonephritis	17	1	1	—	2	21
Chronic pyelonephritis?	24	1	—	—	—	25
Cystitis or urinary tract infection	31	9	2	—	—	42
Significant bacteriuria	25	6	—	—	—	31
Nephrolithiasis	3	1	—	—	—	4
Renal tumour	1	—	—	—	—	1
Urinary tract malformation	8	1	—	—	—	9
Number examined	525	92	12	3	2	634

Haematuria Twenty eight (4 4°) of the 634 clinically examined women had haematuria according to the definition given under Material In three of these 28 the Albustix reading was positive once and in one it was positive in all four tests The remaining 24 (86) were throughout Albustix negative The frequency of haematuria was higher in the age groups above 29 than in the age groups 15–29 (6 8 as against 1 7 % $p < 0\ 005$)

Diagnosis The diagnoses which were made by the examining physician after the investigation are recorded in Table IV The two cases in which the Albustix readings were positive throughout were both diagnosed as chronic pyelonephritis One of these patients had also hypertension Of the three patients with three positive Albustix readings two were subjected to a more comprehensive nephrological investigation including renal biopsy which showed no abnormalities The third who was investigated as an outpatient had a history of urinary tract infection but apart from proteinuria the clinical examination showed nothing abnormal

In the group with negative Albustix readings throughout the diagnosis was status post glomerulonephritidem in 6 hypertension in 26 and suspected or probable urinary tract infection or pyelonephritis in 72 cases

In 4 of 21 cases diagnosed as pyelonephritis the Albustix readings were positive in one or more tests

2 *Sulphosalicylic acid test*

Frequency of positive results (Table V) Of the 412 women who attended the clinical examination 48 (11 7) had been positive in the sulphosalicylic acid test at the screening examination 103 (25 0 %) doubtfully positive and 261 (63 3°) negative

Of the 48 women who were positive at the screening examination 14 6 % were positive at the next two examinations while of the 261 women who were negative at the screening 1 9 were positive in both and 19 1 in one of the two tests made at the clinical examination In the group with doubtfully positive results at the screening the frequency of positive results at the clinical examination was on the whole the same

Table V *Results of the two sulphosalicylic acid tests at the clinical examination in the groups with negative intermediate and positive results at the screening examination*

0=negative (+)=intermediate +=positive

Sulphosalicylic acid test screening	Number examined	Results of the two sulphosalicylic acid tests at the clinical examination 00 ( )	0(+) ( )	(+)(+) ( )	0+ ( )	(+)+ (%)	++ ( )
Negative	261	58	0 7	—	18 4	0 7	1 9
Intermediate	103	44 7	7 8	[illegible]3 3	19 4	1 9	2 9
Positive	48	9	0 8	—	39 6	[illegible]1	14 6

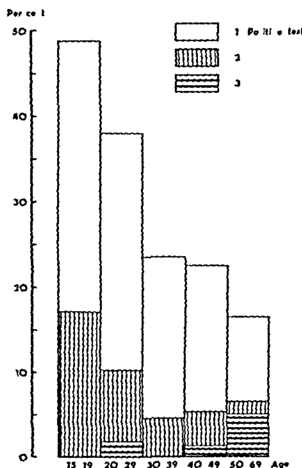


Fig 4 Frequency of positive sulphosalicylic acid tests on 1 and 3 occasions in 41? women

as in the group of women who were negative at the screening

Accordingly 7 women (1 7 %) were positive on all three occasions (including the screening examination) 28 (6 8 %) were positive on two occasions and 92 (22 3 %) on one occasion whereas the remaining 285 (69 2 %) were negative throughout

Age The frequency of intermittently positive cases fell significantly with increasing age ($p <$ 0 0005) (Fig 4)

Diagnosis (Table VI) Two of the seven cases which were throughout positive in the sulphosalicylic acid test were diagnosed at the clinical examination as chronic pyelonephritis One of these seven women had also hypertension The other five showed no other evidence of renal or urinary tract disease In the group with negative sulphosalicylic acid tests three cases were diagnosed as status post glomerulonephritidem 13 as hypertension and 47 as suspected or probable urinary tract infection or pyelonephritis Among the 11 cases assessed as chronic pyelonephritis the sulphosalicylic acid test was positive in 6 on one or more occasions

DISCUSSION

In order to assess the clinical value of tests for proteinuria we must first be able to define the criteria of proteinuria But opinions differ on this point (10 26 35) probably because the investigations are made under different conditions on different materials and with the use of different methods for protein determination The importance of the latter fact is illustrated by the discrepancy between the Albustix and the sulphosalicylic acid tests in the present study

The Albustix test is a paper strip test based upon the protein error of tetrabromophenol blue The strip is impregnated with the dye which is buffered to a pH of approximately 3 0–3 5 At this pH the amino groups of the urine proteins react with the dye and change the colour from yellow to green–blue As albumin has relatively more amino groups than have globulins and mucoproteins the kind of urinary proteins must be considered in the evaluation of the test The Albustix test is for example not a reliable method for indicating M-components in the urine (3 10 37)

Since it was first introduced (1 6 7) the test has been widely used both in clinical practice and in health surveys Free after his studies credited the test with high specificity and sensitivity But in later reports (33 34) its

Table VI *Frequency of the different clinical diagnoses in relation to the results of the three sulphosalicylic acid tests*

Diagnosis	Results of the sulphosalicylic acid test				
	000	00+	0++	+++	Total
Status post glomerulonephritidem	3	1	—	–	4
Hypertension	13	4	1	1	19
Chronic pyelonephritis	11	?			17
Chronic pyelonephritis	14	3	1		18
Cystitis or urinary tract infection		13	3	–	35
Significant bacteriuria	9	9	6		4
Nephrolithiasis	1	1	1	–	3
Renal tumour	1	–	—		1
Urinary tract malformation	4	1	—		5
Number examined	290	9	8		417

value has been questioned, because it has been shown that factors other than the protein concentration influence the results of the test

Even if it has been credited with greater reliability than the Albustix test the sulphosalicylic acid test also involves some sources of error Turbid urine and the presence of mucoproteins may for instance cause false positive results

The evaluation of a single test is also made difficult by the fact that even in normal persons the urinary excretion of protein will often increase during stress and physical activity (11 27 30) Moreover the concentration of the urine must also be taken into consideration The time of sampling is therefore in general of significance in the evaluation of the result

The high proportion of women with a history of proteinuria (17 2) in our material should hardly be considered surprising since the investigated group had had numerous opportunities of having their urines examined for instance at health controls during and after school age examinations to obtain health certificates and pregnancy tests As regards proteinuria in the history its clinical significance is difficult to assess one reason being as was mentioned above that different examiners use different criteria of proteinuria Moreover in our series of women it was difficult to know whether the proteinuria had been intermittent or persistent

The higher proportion of women with a history of proteinuria among those who had borne children as compared with the nulliparae (20 3% as against 14 2) was expected not only because proteinuria is a common complication of pregnancy but also because pregnant women undergo careful testing of their urines The marital status seems to be of no significance as the proportion was the same for married and unmarried nulliparae

It is reasonable to presume that the personnel of a big hospital would have greater possibilities of being examined than would those working in small institutions and this is probably the explanation of the difference in the proportion of women with a history of proteinuria between these two groups although other causes cannot be excluded

As far as we can find the literature contains no report of an investigation that is wholly comparable with the one presented here as among other factors our material can be regarded as selected (hospital personnel) Yet we found in the screening examination the same correlation between the frequency of proteinuria and age as that found by Thysell (1968 unpublished data) in a public health survey and by others in investigations of men (32)

The results of the proteinuria tests obtained on the different occasions showed surprisingly poor agreement Thus two of the 46 women who were Albustix positive in the screening examination were positive in the three subsequnt tests whereas 33 were subsequently negative throughout For the sulphosalicylic acid test the agreement seemed to be somewhat better

Wolman (36) investigated 110 healthy physically active young men on eight occasions in the course of five days 60% of them had proteinuria on one or more occasions most of them (56%) were positive once only In our selected material of women who were examined clinically we found that no less than 70 7% of the 634 women were doubtfully positive or positive and 17 2% clearly Albustix positive in at least one of the four urine tests for proteinuria For the sulphosalicylic acid test which was performed on only three occasions similar figures were noted Wolman s and our observations show how precarious it is to evaluate a single positive test for proteinuria

The clinical examination showed that the higher frequency of proteinuria in the younger age groups noted at screening represented in the main intermittent or transient proteinuria The genesis and the clinical significance of this form of proteinuria which has been described by various attributes (intermittent physiological transient periodic postural orthostatic orthotic lordotic cyclic juvenile) have been the subject of many studies (3 4 8 12 13 15 16 17 19 21 27 36) The increased excretion of protein has been ascribed to various causes for instance haemodynamic endocrine and emotional factors

Physical activity and stress can also cause increased protein excretion in healthy persons with resulting positive routine tests for proteinuria (19 23 27 30 36) and it is quite conceivable that greater physical activity in the younger age groups of a material like that presented here could explain in part the age variation for intermittent proteinuria It is possible that a careful analysis of the single protein components could yield more detailed information (23 24)

As mentioned above mucoproteins can give false positive proteinuria tests (6 18 33) and pyuria and contamination by vaginal secretion among other factors can therefore be sources of

error in a female patient material We found indeed that the frequency of pyuria was significantly higher in the groups with intermediate and positive Albustix or sulphosalicylic acid tests at the screening examination but as it was not correlated with age this potential source of error could not have explained the age variation for positive protein tests Theoretically the sulphosalicylic acid test should be more sensitive for mucoproteins than the Albustix test We did not find any difference between the two tests however with respect to pyuria

The specimen can also give a false positive Albustix reading if the urine is alkaline and/or has a high buffer capacity But in the present material there were no age variations in urinary pH which might account for the high proportion of positive Albustix tests in the lower age groups The possibility that the urines of the younger women had a higher buffer capacity cannot be excluded however On the other hand the same correlation with age was found for the sulphosalicylic acid test as for the Albustix test

King (13) in an investigation of young men with various forms of proteinuria noted diastolic hypertension (above 90 mm Hg) in 15% of the men with orthostatic proteinuria No such tendency was noted in our material We found instead that the diastolic pressure was significantly lower in the age group 30–39 of women with one positive Albustix test than with negative Albustix tests throughout

A tendency to lower body weight has been noted in orthostatic proteinuria (4 32) We found no difference in body weight height or the ratio weight/height between the women who had intermittent proteinuria and those whose urines were throughout Albustix negative

It has long been known (6) that microscopic haematuria may be present without proteinuria. This was verified in the present study in which we found that only four of 28 women with microscopic haematuria were Albustix positive on some occasion

As regards the clinical significance of the intermittent proteinuria opinions have changed Earlier the condition was regarded as quite harmless but a more expectant and variable attitude has now been adopted Follow up studies of large series of patients have shown that the intermittent proteinuria not seldom develops into a persistent form and may sometimes represent a forerunner of renal disease On examination of biopsy specimens organic renal changes have in fact been demonstrated in some cases (20 22 28 29)

In some cases of asymptomatic intermittent proteinuria clinical investigations (3 17 36) biopsies (8 20 22 28) or follow up studies (4 5 12, 16 17 32) have shown that this form of proteinuria may represent diverse pathological conditions such as pyelonephritis glomerulonephritis nephrolithiasis hypertension hypercalcaemia hypopotassaemia hyperuricaemia diabetes urinary tract malformations and the Fanconi syndrome In our series the clinical assessment of intermittent proteinuria led to a clinical diagnosis (status post glomerulonephritidem urinary tract infection pyelonephritis nephrolithiasis hypertension and urinary tract malformations) in 23% (Albustix) and 28% (sulphosalicylic acid test) respectively while the rest of the investigated women with this kind of proteinuria were regarded as healthy

As regards the persistent proteinuria its clinical significance has been considered to differ from that of the intermittent proteinuria (3 5 12 13) The two women with positive Albustix readings throughout in the present study were clinically assessed as having chronic pyelonephritis One of them had also hypertension The same women were also positive in the three sulphosalicylic acid tests but there were five others with positive sulphosalicylic acid tests throughout who had no other signs of urinary tract disease

It is well known that urinary tract disease can be present without demonstrable proteinuria, which was verified in the present study We found that about one fifth of the women who had negative proteinuria tests throughout had some form of urinary tract disease or hypertension Thus it seems that screening for proteinuria is not sufficient as the sole method for the detection of renal disease in health surveys

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BLOOD LEVELS OF INSULIN IN URAEMIC PATIENTS

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Abstract Oral glucose tolerance and intravenous tolbutamide tests have been performed on 11 uraemic patient with normal fasting blood sugar on 9 non uraemic patients with normal oral glucose tolerance and on 13 non uraemic patients with abnormal glucose tolerance but with normal fasting blood sugar At the same time the blood level of insulin was determined by an immunological technique The glucose response of the uraemic patients during glucose tolerance as well as during tolbutamide tests did not differ from the average response in the group of non uraemics On the other hand, the insulin response was increased in the uraemic patients during both tests The uraemic patients also exhibited an increased insulin response to tolbutamide as compared with the 13 non uraemic patients with abnormal glucose tolerance

Several authors have demonstrated impaired glucose tolerance by oral glucose tolerance tests in uraemic patients (5 6 10 11) However in uraemic patients with a serum creatinine concentration exceeding 4 mg per 100 ml compared with control persons Andersen and Friis (3) found a significant difference in the two hour value only in patients below 60 years of age

Attempts have been made also to elucidate the reaction to the tolbutamide test (14) in uraemic subjects Ahlers et al (1) found an abnormal reaction whereas Westervelt and Schreiner (15) and Tchobroutsky et al (13) reported normal findings The latter authors found a normal insulin level in the blood of uraemic patients but a delayed increase during tests causing hyperglycaemia According to criteria set up in a previous paper (2) Andersen and Friis (3) found a significantly increased number of abnormal tolbutamide tests in a series of uraemic patients when the serum creatinine exceeded 4 mg per 100 ml but only in patients under 60 years of age Hampers et al (7) studied five patients with severe uraemia before and after haemodialysis They found reduced sensitivity to insulin abnormal i v glucose test abnormal tolbutamide test and reduced response of serum insulin to i v glucose All these reactions returned to normal after haemodialysis Briggs et al (4) have studied 12 uraemic patients and 14 controls In their material too the oral glucose tolerance was reduced the fall in blood sugar being delayed Plasma insulin behaved much like the blood sugar showing also a delayed fall Lastly Hutchings et al (9) studying 12 uraemic patients with a creatinine clearance below 4 8 ml/min found the glucose tolerance to be reduced in the majority while the insulin values increased more than in normal subjects during i v glucose tolerance tests

PRESENT INVESTIGATIONS

Material

In order to assess the serum insulin concentration during oral glucose tolerance and intravenous tolbutamide tests in uraemic patients without diabetes and with a normal fasting blood sugar (< 110 mg/100 ml) the following three groups of patients were investigated

A 11 uraemic patients with normal fasting blood sugar

B 9 non uraemic patients with normal oral glucose tolerance

C 13 non uraemic patients with abnormal glucose tolerance but with a normal fasting blood sugar

As uraemic patients we consider patients with a renal disease and a serum creatinine exceeding 1 5 mg/100 ml determined by autoanalyzer (Technicon)

The age distribution of the three groups was as follows

A 61 6 ± 8 4 y (43 78 y 7 over 60)
B 48 5 ± 19 0 y (17–71 y 4 over 60)
C 54 9 ± 18 7 y (? –85 y 6 over 60)

Table I *Clinical data for 11 patients with renal failure*

Case no	Sex	Age	Diagnosis	Serum creatinine (mg/100 ml)	Serum urea (mg/100 ml)	BP (mm Hg)	Height (cm)	Weight (kg)
1	♀	61	Chronic pyelonephritis	1 5	65	160/100	16?	6?
2	♀	43	Chronic pyelonephritis	3 9	81	130/90	168	57
3	♀	64	Chronic pyelonephritis	4 0	6?	150/90	156	42
4	♀	64	Chronic pyelonephritis	4 1	119	180/110	158	56
5	♀	69	Chronic pyelonephritis	4 2	90	150/95	157	63
6	♂	7?	Chronic pyelonephritis	4 5		130/70	168	77
7	♂	63	Chronic pyelonephritis	4 7	111	180/100	180	9?
8	♀	56	Chronic pyelonephritis	5 2	109	130/90	165	69
9	♀	70	Chronic pyelonephritis	5 7	92	135/70	159	5?
10	♀	62	Chronic pyelonephritis	8 0	144	?20/110	158	64
11	♀	53	Polycystic kidneys	15 6		140/100	156	47

There was no statistically significant difference between the age distribution in the three groups (between A and B $t = 1\,98$ $0\,1 > P > 0\,05$ and between A and C $t \sim 0\,73$ $P > 0\,1$)

No patient was obese

The serum creatinine concentration in the uraemic patients was 1 5–15 6 mg/100 ml mean 4 67 mg/100 ml Nine had a concentration exceeding 4 0 mg/100 ml All but one were normotensive and all had chronic pyelonephritis except one who had polycystic kidneys

Methods

Glucose tolerance as well as tolbutamide tests were performed in the morning after fasting overnight

The glucose tolerance test consisted in the administration of 1 g anhydrous glucose per kg body weight the maximum dose being 70 g Ear blood was drawn for determining the blood level of glucose and venous blood for determining the serum insulin immediately before 15 30 60 90 120 150 and 180 min after the intake of glucose The tolerance test was considered normal if the 2 hour blood glucose level was less than 130 mg/100 ml (cf Andersen and Friis (2))

The tolbutamide test was performed by intravenous injection of 1 g sodium tolbutamide in the course of 1 min Ear blood was drawn for glucose determination and venous blood for insulin determination immediately before and 10 20 30 60 and 90 min after the injection. Moreover venous blood was drawn 5 min after the injection According to the criteria set up by Andersen and Friis (?) the response was considered normal when the ?0 min blood glucose concentration was less than 80 of the initial value or when the 30 min sample showed the minimum concentration On the other hand it was considered slightly abnormal when the ?0 min concentration was ≥ 80 of the initial value and the 60 min sample showed the minimum. The test was interpreted as severely abnormal when the minimum was not reached in 90 min

Blood sugar was determined enzymatically by hexokinase and glucose-6 phosphate-dehydrogenase (1?)

Serum insulin was determined by radioimmunoassay (8) free and antibody bound insulin being separated by alcohol precipitation (The Novo Research Institute supplied human standard insulin hog ^{125}I insulin and insulin antibody (serum from guinea pigs immunized by hog insulin) for this study) Triple determinations were carried out and the coefficient of variation was approx. 10 and approx 6 at concentrations of ?0 and 60 μU/ml respectively

Table II *Blood glucose and serum insulin concentration during oral glucose tolerance test in 11 patients with renal failure*

Case no	Glucose in blood (mg/100 ml) (h) 0	1	2	3	Serum insulin (μU/ml) (h) 0	1	2	3
1	89	240	1?4	5	15	180	132	8
2	84	181	103	51	10	99	60	26
3	104	?41	148	77	8	54	40	29
4	96	225	115	84	12	142	38	16
5	113	?01	121	104	31	250	88	56
6	104	168	176	113	23	36	78	51
7	87	227	?07	153	0	93	136	10?
8	87	119	90	74	7	83	38	36
9	81	214	168	76	27	137	80	36
10	107	185	117	102	16	162	144	126
11	79	164	148	108	0	98	91	56
Mean	94	197	138	91	14	1?1	84	51
S D	12	38	35	34	10	58	39	34

RESULTS

The clinical data for 11 patients with varying degrees of renal failure are shown in Table I while Tables II and III give blood glucose and insulin concentration during glucose tolerance and tolbutamide tests One patient (case 10) had hy

Table III *Blood glucose and serum insulin concentration during intravenous tolbutamide test in 11 patients with renal failure*

Case no	Glucose in blood (mg/100 ml) (min) 0	10	20	30	60	90	Serum insulin (μU/ml) (min) 0	5	10	20	30	60	90
1	91	77	64	53	61	66	16	100	107	87	83	2	11
2	87	74	66	48	61	70	7	51	57	43	32	15	8
3	90	83	80	77	60	65	7	7	‿5	3	15	11	14
4	91	89	76	67	47	55	8	75	89	93	81	23	‿0
5	105	98	89	80	67	74	33	139	151	140	85	36	8
6	100	91	91	85	65	69	16	53	56	43	9	1	10
7	93	97	84	82	70	69	11	55	58	45	‿5	9	7
8	83	76	50	30	40	54	18		166	160	86	24	16
9	79	75	66	59	40	46	27		70	63	63	33	‿6
10	98	97	80	69	46	52	15	86	83	90	70	30	18
11	83	77	65	49	46	50	9		60	65	50	34	27
Mean	91	84	74	64	55	61	15	73	84	78	56	23	17
S.D.	8	9	13	17	11	10	8	35	43	42	27	10	8

pertension The glucose tolerance tests were normal in five cases (nos 1 2 4 5 and 10) The serum insulin value in the fasting patients averaged 14±10 μU/ml ranging from 0–31 μU/ml At the end of 1 hour a maximum of 36–250 μU/ml was reached and at 3 hours the value ranged from 16 to 126 μU/ml As far as the tolbutamide tests were concerned six patients were found to have slightly abnormal responses (cases 3 4 5 6 9 and 10) and one a severely abnormal one (case 7) Serum insulin generally reached a maximum of between 25 and 166 μU/ml 10 min after the injection of tolbutamide in order to fall gradually thereafter and return to the initial level in 90 min

For comparison the findings in the nine non uraemic patients with normal glucose tolerance are as follows clinical data listed in Table IV blood glucose and insulin concentrations during glucose tolerance and tolbutamide tests in Tables V and VI The fasting insulin levels averaged 16±5 μU/ml ranging from 10 to 25 μU/ml i e as in the uraemic subjects rising to 41–154 μU/ml at 1 hour and thereafter returning to the initial values in another 2 hours

In the tolbutamide test cases 1 2 4 5 and 8 reached their minimum glucose values in 30 min i e the response was normal In the other four it was slightly abnormal In merely 5 min the insulin had reached the highest recorded value ranging from 37–80 μU/ml then falling gradually and returning to the initial level in 90 min As far as the course of the insulin concentration is concerned there was no difference between patients with normal and with abnormal tolbutamide response

Tables VII–IX lastly illustrate the findings in 13 non uraemic patients with abnormal glucose

Table IV *Clinical data for 9 non uraemic patients with normal oral glucose tolerance*

Case no	Sex	Age	Diagnosis	BP (mm Hg)	Height (cm)	Weight (kg)
1		46	Duodenal ulcer	1 0 80	160	50
3	o	17	Colitis	110 70	178	72
4		47	Cardiac neurosis	1 5 70	161	53
5	o	42	Gastritis	1 0 90	166	60
6	o	60	Cholelithiasis	150 90	159	64
7		60	Lipothymia	110 80	159	54
8	o	41	Enteritis	1 0 80	176	76
9	o	53	Bronchitis	140 90	161	64
		71	Duodenal ul-er	145 0	151	8

Table V *Blood glucose and serum insulin concentration during oral glucose tolerance test in 9 non uraemic patients with normal glucose tolerance*

Case no	Glucose in blood (mg/100 ml) (h) 0	1	2	3	Serum insulin (μU/ml) (h) 0	1	2	3
1	74	227	114	55	16	74	15	0
2	86	99	102	76	25	52	67	25
3	91	211	117	58	10	77	68	9
4	80	144	59	74	12	56	19	13
5	92	149	124	99	12	58	29	9
6	86	193	119	82	20	79	44	27
7	87	159	124	82	23	154	81	28
8	77	126	99	51	15	41	28	10
9	85	227	119	66	12	114	59	18
Mean	84	171	109	71	16	78	46	15
S D	6	46	21	16	5	35	24	10

tolerance but with a normal fasting blood sugar i e patients with latent diabetes One (case 8) had hypertension Fasting insulin ranged from 0–28 μU/ml and during the glucose tolerance it reached a maximum of 38–198 μU/ml in 2 hours at 3 hours it ranged from 2 to 157 μU/ml The tolbutamide tests were slightly abnormal in cases 1 3 4 6 7 8 9 and 13 and severely abnormal in case 2 In this group the serum insulin reached as an average a maximum of 23–142 μU/ml 10 min after injection of tolbutamide and thereafter gradually returned to the initial levels in 90 min

The course of the serum insulin level showed practically no difference between patients with normal and with abnormal tolbutamide tests

The mean values for the three groups of patients are shown in Figs 1 and 2

Table VI *Blood glucose concentration and serum insulin during intravenous tolbutamide test in 9 non uraemic patients with normal oral glucose tolerance*

Case no	Glucose in blood (mg/100 ml) (min) 0	10	20	30	60	90	Serum insulin (μU/ml) (min) 0	5	10	20	30	60	90
1	78		66	51	53	66	10		45	51	40	[illegible]	66
2	83	79	70	64	68	68	19	69	58	35	38	18	76
3	88	84	72	63	47	66	11	52	44	36	30	18	12
4	79	70	58	51	63	66	14	37	36	35	35	26	6
5	92	89	73	58	70	71	13	80	61	41	31	16	17
6	88	86	80	67	54	68	13	78	85	83	53	30	3
7	83	80	72	62	61	71	11	70	64	65	46	[illegible]	17
8	73	69	49	39	56	62	7	48	44	29	8	6	5
9	87	84	75	69	59	67	16	66	64	48	34	22	15
Mean	83	80	68	58	59	67	13	63	56	47	35	0	19
S D	6	7	9	10	7	3	4	15	13	18	13	7	9

Table VII *Clinical data for 13 non uraemic patients with abnormal oral glucose tolerance*

Case no	Sex	Age	Diagnosis	BP (mm Hg)	Height (cm)	Weight (kg)
1	♂	29	Colitis	110 70	187	83
2	♀	66	Erythema nodosum	120/80	165	55
3	♀	41	Melaena	115 60	159	58
4	♀	53	Colitis	130 80	164	68
5	♀	32	Constipation	115/75	160	5
6	♂	22	Constipation	115 65	170	59
7	♀	59	Hodgkin s disease	1 0/80	160	0
8	♀	85	Cerebral arteriosclerosis	180/1 0	159	67
9	♂	67	Pulmonary malignancy	130/70	168	50
10	♂	54	Periarthrosis humero scapularis	175/110	170	80
11	♀	67	Colitis	195/100	16	69
12	♀	70	Cerebral arteriosclerosis	130/70	161	57
13	♀	68	Osteoarthritis	120 70	160	61

Table VIII *Blood glucose and serum insulin concentration during oral glucose tolerance test in 13 non uraemic patients with abnormal glucose tolerance*

Case no	Glucose in blood (mg/100 ml) (h) 0	1	2	3	Serum insulin (μU/ml) (h) 0	1	2	3
1	88	216	150	61	18	48	45	11
2	88	159	131	64	9	62	39	9
3	86	176	153	63	9	1?8	151	34
4	9?	244	181	64	0	108	78	25
5	100	182	148	86	4	34	38	2
6	90	179	144	86	13	56	56	28
7	96	?04	146	81	21	52	43	21
8	92	216	234	226	19	80	150	157
9	8	171	150	102	12	78	100	42
10	78	2 0	210	120	28	165	198	126
11	90	2 0	182	104	18	85	115	32
12	88	231	?07	91	19	129	94	26
13	77	182	132	43	4	49	40	6
Mean	88	200	167	92	13	83	88	41
S.D	7	26	33	46	7	39	53	47

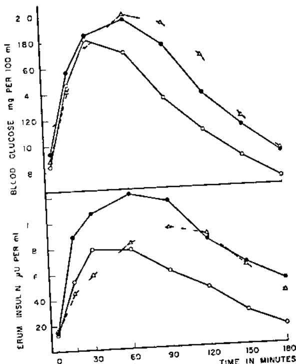


Fig 1 Oral glucose tolerance test • group A uraemics O group B normals ∠ group C patients with normal fasting blood sugar but abnormal glucose tolerance test *Abscissa* time in minutes *Ordinate* blood glucose mg/100 ml serum insulin μU/ml

Fig 1 illustrates the findings during glucose tolerance A comparison of the individual groups shows

1 *Normal subjects versus non uraemic patients with abnormal glucose tolerance*

The glucose values differed significantly at 1 1/? and 2 1/ hours ($P<0.001$ $P<0.001$ and $0.02>P>0.01$) while the fasting values and the 1 and 3 hour values did not differ The insulin values also showed a difference at 2 hours

Table IX *Blood glucose and serum insulin concentration during intravenous tolbutamide test in 13 non uraemic patients with abnormal oral glucose tolerance*

Case no	Glucose in blood (mg/100 ml) (min) 0	10	0	30	60	90	Serum insulin (μU/ml) (min) 0	5	10	0	30	60	90
1	80		69	61	61	69	1?		26	14	15	9	11
2	94		79	75	62	59	2		35	36	29	15	13
3	88	88	79	69	56	71	12	94	99	84	47	11	9
4	85	83	75	66	52	62	10	31	27	7	0	11	7
5	89	80	64	51	69	70	0	28	9	25	5	0	0
6	91	85	77	65	57	68	19	38	28	37	?7	13	17
7	93	96	85	74	55	63	0	53	41	40	7	19	0
8	99	96	94	83	73	61	2	19	3	19	17	11	4
9	87	85	77	70	58	67	18	55	5	4	34	18	17
10	83	72	58	47	53	61	27	153	14	91	56	31	8
11	9	81	70	56	57	65	1	70	75	58	37	17	11
12	82	75	64	55	46	55	?3	85	75	49	38	4	26
13	75	70	6	5?	47	51	7	31	36	27	18	10	8
M an	88	83	72	64	57	64	13	51	53	4	8	15	13
S D	7	8	10	11	8	6	8	38	36	23	14	8	8

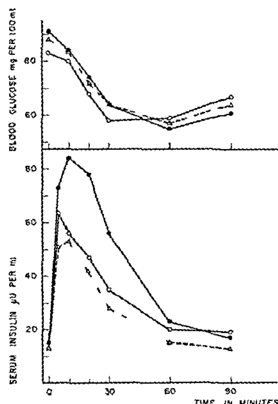


Fig 2 Intravenous tolbutamide test ● group A uraemics O group B normals △ group C patients with normal fasting blood sugar but abnormal glucose tolerance test *Abscissa* time in minutes *Ordinate* blood glucose mg/100 ml serum insulin μU/ml

(0 05 > *P* > 0 02) the patients showing the highest ulin values The insulin curves are seen to run most parallel to the glucose curves

2 Uraemic patients versus normal subjects

Fasting 1 1/2, 2 and 2 1/2 hour glucose values were significantly higher in the uraemic than in the normal subjects (0 05 > *P* > 0 02, 0 01 > *P* > 0 001, 0 05 > *P* > 0 02 and 0 05 > *P* > 0 02) Among the insulin values the 1 1/2, 2 and 3 hour values were higher in the uraemic patients (0 02 > *P* > 0 01, 0 05 > *P* > 0 02 and 0 01 > *P* > 0 001)

3 Uraemic patients versus non uraemic patients with abnormal glucose tolerance

The 2 hour glucose values for the uraemic patients were significantly *lower* than for the other group (0 05 > *P* > 0 02) While the insulin values showed no significant difference at 2 hours the 30 min sample showed significantly higher levels in the uraemic patients (0 02 > *P* > 0 01)

Fig 2 presents the findings during tolbutamide tolerance A comparison between the individual groups shows

1 Normal subjects versus non uraemic patients with abnormal glucose tolerance

Neither the glucose nor the insulin values showed any significant difference between the two groups The only recorded difference was that the insulin level reached a maximum 5 min after the injection of tolbutamide in the normal subjects and not until 10 min after the injection in the other group

2 Uraemic patients versus normal subjects

The glucose values in these two groups did not differ except for the fasting values which were significantly higher in the uraemic patients (0 05 > *P* > 0 02) The 20 and 30 min insulin values were higher in the uraemic patients (0 05 > *P* > 0 02) while the 10 min values showed no significant difference (0 1 > *P* > 0 05)

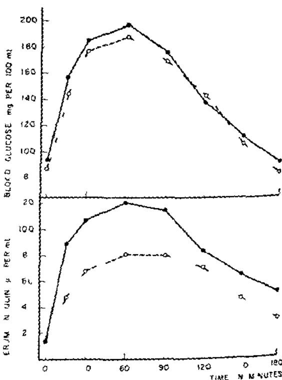


Fig 3 Oral glucose tolerance test ● uraemics o non uraemics *Abscissa* time in minutes *Ordinate* blood glucose mg 100 ml serum insulin μU/ml

3 Uraemic patients versus non uraemic patients with abnormal glucose tolerance

While the glucose values in the two groups did not differ the 10 20- 30 and 60-min insulin values were significantly higher in the uraemic patients ($0.05 > P > 0.02$ $0.02 > P > 0.01$ $0.01 > P > 0.001$ and $0.05 > P > 0.02$)

If all non uraemic subjects are considered together the mean curves are as shown in Figs 3 and 4 It is apparent from Fig 3 that the glucose levels during glucose tolerance tests are identical with the exception of the fasting values which are somewhat higher among the uraemic patients ($0.05 > P > 0.02$) Serum insulin was significantly higher in the uraemic patients at $^1/_2$, 1 and $1\,^1/_2$ hours ($0.02 > P > 0.01$ $0.01 > P > 0.001$ and $0.05 > P > 0.02$) From Fig 4 it is apparent that the glucose values during the tolbutamide test were also identical for both groups while the serum level of insulin was significantly higher in the uraemic patients at 10 20 and 30 min ($0.05 > P > 0.02$ $0.01 > P > 0.001$ and $0.01 > P > 0.001$) The maximum was not reached until at 10 min while the non uraemic subjects reached their maximum in 5 min

Lastly an analysis was made for correlation between the height of the serum creatinine and the increase in serum insulin during the tolbutamide test No such correlation seemed to exist and the hypertensive uraemic patient did not exhibit a greater increase of insulin than the others but the increase persisted in spite of the decrease in blood glucose

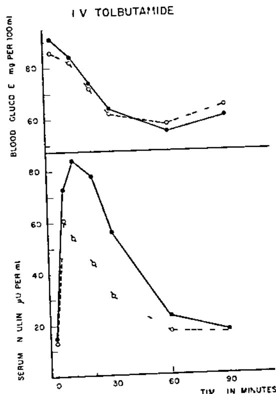


Fig 4 Intravenous tolbutamide test ● uraemics O non uraemics *Abscissa* time in minutes. *Ordinate* blood glucose mg/100 ml serum insulin µU/ml

DISCUSSION AND CONCLUSION

Comparison of the glucose values found in the 11 uraemic and in the 22 non uraemic patients during oral glucose tolerance as well as during tolbutamide tests failed to show a significant difference These findings are not in agreement with a number of previous studies (1 6 10 11) but are confirmed by Westervelt and Schreiner (15) as well as by Tchobroutsky et al (13) who demonstrated normal tolbutamide response in uraemic subjects In a previous study of a large material Andersen and Friis (3) found no difference in glucose tolerance or tolbutamide tests between uraemic and non uraemic patients except in those who were under 60 years of age and had a serum creatinine exceeding 4 mg/100 ml The present series is too small for any similar conclusions

Our finding that the insulin concentration which in the fasting state is as in normal subjects increases significantly more in uraemic than in non uraemic patients during oral glucose tolerance and tolbutamide tests is at variance with Tchobroutsky et al (13) who found in uraemic patients a delayed insulin response to tests causing hyperglycaemia Our findings also disagree with those of Briggs et al (4) who reported a delayed fall in the insulin concentration during oral glucose tolerance

A comparison of the insulin concentration in the uraemic patients and in the 13 non uraemic patients with abnormal glucose tolerance shows that the insulin response of the uraemics was stronger during the tolbutamide test while during the glucose tolerance test only the 30 min values were higher In the uraemic patients the maximum increase after oral glucose occurred at 60 min and during the tolbutamide test at 10 min the

corresponding figures for the 13 non uraemic patients were 90 and 10 min The nine non uraemic patients with normal glucose tolerance reached on the average the insulin maximum in the glucose and tolbutamide tests at the end of 30 and 5 min respectively The increase after oral glucose was less marked than in the 13 non uraemic patients with abnormal glucose tolerance

The investigations indicate that the insulin response during the glucose tolerance and particularly during the tolbutamide test is increased in uraemic compared with non uraemic persons even though the latter have abnormal glucose tolerance The explanation of this phenomenon may be that these patients have circulating substances which counteract the insulin or that their tissues are less sensitive to the action of insulin

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IRON RESISTANT HYPOCHROMIC ANEMIA IN A SCANDINAVIAN FAMILY HETEROZYGOUS β-THALASSEMIA

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Abstract Four cases of heterozygous β thalassemia in three generations of a Norwegian family are reported. Hypochromic microcytic anemia with many target cells and a high serum iron concentration suggested the diagnosis hemoglobin starch-gel electrophoresis demonstrating an increased Hb A fraction and family studies confirmed it. It is concluded that iron resistant hypochromic anemia in individuals of apparently Scandinavian descent may be due to β thalassemia

Thalassemia is a group of hereditary disorders of hemoglobin synthesis There are two main types α and β thalassemia resulting from a defect in the production of either α or β chains of globin In β-thalassemia the synthesis of adult hemoglobin (Hb A) is inhibited while the normally occurring Hb A and Hb F fractions may be increased No abnormal hemoglobin is formed The hypochromic and microcytic anemia of thalassemia results from a combination of defective hemoglobin synthesis and a shortened red cell survival

This genetic abnormality occurs predominantly in the Mediterranean area in the Far East and in Negroes However an increasing number of cases has been discovered in North European populations (1 2 3 5 7 10) In 1958 Ytrehus (15) suspected the disease in two siblings of Norwegian-German parentage a diagnosis we have confirmed later by starch gel electrophoresis The recent report of two Swedish families with the β thalassemia trait described the first cases among people of apparently Scandinavian descent (9) In this article we report four cases of heterozygous β thalassemia in a Norwegian family and discuss the problems of diagnosis

METHODS

Routine hematological investigations were done by standard laboratory methods Osmotic fragility of the red cells was investigated as described by Parpart et al (8) slightly modified (4) Hemoglobin solutions were prepared according to Dacie and Lewis (4) and adjusted to the same Hb level. Starch gel electrophoresis was carried out as described by Smithies (13) in Tris buffer (6) at pH 8 6 Hb A was reported normal or increased by visual comparison with control specimens Fetal hemoglobin was determined by the method of Singer et al. (11)

RESULTS

Report of Cases

Fig 1 shows that the thalassemia trait was found in all living generations of the family

The propositus (B 1) was a 40 year-old man A mild hypochromic anemia was first discovered when he was 20 years old Two years later he was admitted to hospital because his anemia did not respond to iron therapy At that time Hb was 12 4 g/100 ml and the serum iron was 170 g % Physical examination was negative In the following years he received numerous courses of oral and intramuscular iron without benefit He was tired but regarded himself as healthy He had never been icteric Physical examination on the day of admission was negative except for areas with skin atrophy and diffuse pigmentation over both malleoli on the left leg and over the medial malleolu on the right leg Varicosities were not present and he had probably never had thrombophlebitis

The 80-year-old father of the propositus (A 1) was the oldest carrier of the trait He originated from the South Eastern part of Sweden No

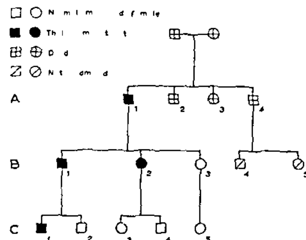


Fig 1 Pedigree of a Scandinavian family with the β thalassemia trait

foreign ancestors could be traced in the family for 150 years He and his only affected grand child a boy aged 15 (C 1) *were totally asymp tomatic* and the physical examination was nega tive

The fourth affected family member (B 2) was the 35 year old sister of the propositus the mother of two healthy children Like her brother she had been diagnosed as mildly anemic at the age of 20 In the following years Hb varied be tween 9 9 and 11 9 g/100 ml the lowest value was recorded during her first pregnancy She [illegible]ved iron orally and parenterally for eight [illegible]ars without effect until she discontinued the medication herself At the time of our investiga tion she felt well *and the physical examination* was normal

Hematological Findings

Table I shows that the two adult men were mildly anemic the woman had a marginal Hb value while the boy s Hb was normal How ever the RBC count was *high in three of the* four affected family members The MCV and MCH *were low in all patients* but MCHC was normal Examination of the peripheral blood *film showed* anisocytosis and poikilocytosis with pencil forms and other bizarre red cell shapes The most striking finding however was the presence of target cells (Fig 2) especially in the anemic patients (Table I) Basophilic stippling of the red cells was occasionally observed The osmotic fragility of the red cells was decreased in all patients (Fig 3) *Serum bilirubin and hapto*globin were normal The reticulocyte count was slightly *elevated Bone marrow aspiration in the* propositus revealed normoblastic erythropoiesis and an increased amount of stainable iron The sideroblasts were normal Serum iron was clearly increased in the propositus in the others it was in the upper normal range (Table I) Leukocytes and platelets were normal

Fig 4 shows a representative example of the Hb electrophoresis which was performed in all family members Although not quantitated in this study the Hb A fraction in the four pa tients was obviously *increased compared with* healthy family members The mean Hb F was 1 7° *only the propositus had a level over* 2% No abnormal Hb components were found

DISCUSSION

Two of the affected family members (A 1 and B 1) presented a triad which is virtually diagnostic of heterozygous thalassemia

1 A hypochromic microcytic anemia with many target cells occasional basophilic stippled red cells pencil and other bizarre red cell shapes Characteristically the morphologic changes in the red cells were far out of proportion to the degree of anemia

Table I *Hematological data of family members with the β thalassemia trait*

Pedigree no	Sex	Age (y)	Hb (g/100 ml)	RBC (× 10⁶/mm³)	MCV	MCH	MCHC	*Retic* ( )	Target *cells* ( )	*Hb F* ( )	Serum *iron* (µg 100 ml)
A1	♂	80	13 3	6 5	63	0	32	2 2	15	1 7	127
B1	♂	40	12 7	5 2	77	24	32	1 8	25	? 5	195
B2	♀	35	11 7	5 5	65	21	33	2 4	12	1 1	1 8
C1	♂	15	14 1	6 6	65	21	33	1 8	2	1 5	115



Fig 2 P nipheral blood film of the propo itu (B 1) showing ani o and poikilocytosis hypochromia target cells and punctate basophilia May Grunwald G emsa stain 780

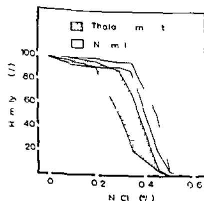
2 Normal or increased serum iron

3 Increased Hb A fraction This is the most discriminative single observation and it is found in the majority of cases with the β thalassemia trait.

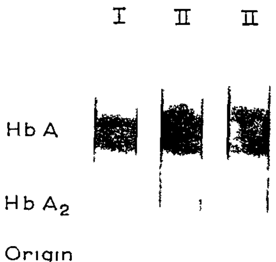
Hb F was slightly increased in the propositus (Table I) values below 2% are considered nor mal However less than 50 % of cases have an increased Hb F fraction

Family studies further confirmed the diagnosis First the hereditary trait of the disease was dis closed consistent with a dominant autosomal transmission Secondly the notable variability of this condition even within a small family group was revealed B 2 and C 1 had all morphologic and biochemical characteristics of the trait, but were not anemic This difference divides the heterozygous state into two clinical forms β thalas semia *minor* with slight to moderate anemia and few symptoms and β thalassemia *minima* which is the asymptomatic trait without anemia

The main differential diagnosis is iron de



F g 3 The decreased osmotic fragility of the red cells f om family members with the β thalassemia trait



F g 4 Starch gel electrophoresis of hemolysates from patients w th the β thala semia tra t (II) and a normal fam ly member (I)

Table II *Hypochromic microcytic anemias*

	Heredity	Target cells	Basophilic stippling	Osmotic fragility	Serum iron
Iron deficiency anemia	–	(+)	(+)	↓	↓
β thalassemia	+	++	+	↓	↑
Sideroblastic anemia	±	(+)	(+)	↓	↑
Lead intoxication	–	–	+++	↓	N
Pyridoxine responsive anemia	–	+	(+)	↓	↑

ficiency anemia, the dominant cause of hypochromic microcytic anemia Iron deficiency anemia is so common in Scandinavia that it would be clearly unrewarding to proceed with a full investigation of the possibility of thalassemia without the preliminary use of a screening test In most cases the response to iron therapy is used to confirm the diagnosis If treatment fails however it is important to make further investigations and consider other diagnostic possibilities (Table II) Unfortunately this is often not done a fact well illustrated by the case stories of patients with β thalassemia Two of the four patients reported here and 11 of the 21 patients in the Swedish investigation (9) had been treated with iron for several years without improvement In iron deficiency anemia the serum iron is low and the Hb electrophoresis is normal Target cells [illegible] cells showing punctate basophilia may be [illegible]ent but they are fewer than in thalassemia A diagnosis of iron deficiency anemia refractory to oral iron therapy must be proved before it is accepted According to Wintrobe (14) these cases are extremely rare

Table II also presents three rare causes of hypochromic microcytic anemia Among them the hereditary type of sideroblastic anemia may easily be confused with thalassemia ("pseudo thalassemia") However in this disease there is no increase in Hb A_2 and abnormal sideroblasts ("ringed sideroblasts") are found in the bone marrow Basophilic stippling of the red cells is the outstanding and characteristic feature in patients exposed to lead Determination of the blood and urinary lead concentration and the copro porphyrin excretion confirm the diagnosis Pyridoxine responsive anemia has many features in common with sideroblastic anemia. Diagnosis rests exclusively on a therapeutic trial

This investigation confirms that β thalassemia exists in apparently aboriginal Scandinavians Unfortunately it cannot be decided whether this is due to a spontaneous mutation or to immigration We can only maintain that foreign ancestors during the last 150 years could not be found and that our family was not related to the two recently reported Swedish families with the β thalassemia trait (12)

An improved diagnostic approach will reveal a number of new cases with thalassemia in Scandinavia in future years The considerable contribution of manpower from Mediterranean countries will promote this development

ACKNOWLEDGEMENTS

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Congress Announcement

The Third International Congress of the Transplantation Society will be held in The Hague September 7 to 11 1970

Secretariat Third International Congress of the Transplantation Society c/o Holland Organizing Centre 16 Lange Voorhout The Hague The Netherlands

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ACUTE EFFECT OF A SUSTAINED RELEASE PYRIDYL CARBINOL PREPARATION RONICOL RETARD® ON PLASMA FREE FATTY ACIDS

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Abstract The effect of two different single doses of a sustained release preparation of pyridyl carbinol Ronicol Retard® taken by mouth was studied in eleven patients The results were compared to previously obtained data with identical experimental procedures for nicotinic acid and placebo

The concentration of free nicotinic acid in plasma rose s owly after 0 45 and 1 05 g of Ronicol Retard® to reach a maximum level after 2 to 3 h and then decl ned to basal levels after 6 or more h

The concentration of FFA in plasma was lowered to the same level as with nicotini acid by both doses of Ron col Retard® In comparison to values obtained after placebo 0 45 g depressed the FFA levels for 1 / h 1 05 g for about 3 h

When the FFA lowering effect vanished there was little rebound (overshoot) of FFA levels after Ronicol Retard® while nicotinic acid gave rise to a pronounced overshoot

Flush similar to that s en after nicotinic acid occurred in all patients receiving 1 05 g of Ronicol Retard® After 0 45 g not all patients showed flush and those who did had it to a slighter degree than after 1 05 g

Compared to nicot n c acid pyridyl carbinol d d not inh bit lipolys s in ad pose tissue when added in vitro unless the concentration was 100 times greater than that of n cotin c acid. Even then the inh bition was much less p onounced than with nicotinic acid Th s find ng togethe with the appearance of free n cotin c acid in plasma after Ronicol Retard® suggests that this compound lowers plasma levels of FFA by being metabolized to nicotinic acid wh ch then inhibits l polysis

The relat onship of lower ng of FFA the FFA o er shoot and othe poss ble effect of nicotinic a d to the lowering of the concent ation of cholesterol in pla ma is briefly d scuss d

In 1955 Altschul and co-workers (1 2) demonstrated that nicotinic acid in high doses lowers the concentration of cholesterol in blood plasma of animals and man It is now evident that nicotinic acid also lowers the other two major plasma lipid fractions contained in the plasma lipoproteins triglycerides and phospholipids (8 12 17) Nicotinic acid is now used with increasing frequency in the treatment of hyperlipoproteinemia

In 1962 we demonstrated that nicotinic acid inhibits the mobilization of free fatty acids (FFA) from adipose tissue (11) It was postulated that this effect was one of several possible mechanisms by which this compound reduces the plasma lipo protein concentration (4 5 14 17 18)

One of the disadvantages of nicotinic acid is that the high doses which are needed to normalize ele vated plasma lipid levels frequently cause side ef fects usually from the gastro intestinal tract (1 13) To reduce these side effects of nicotinic acid preparations with prolonged action like aluminium nicotinate have been tried (1) However it was not possible to abolish the side effects of nicotinic acid with these early preparations

Several compounds chemically related to nico tinic acid have been shown to inhibit the mobiliza tion of FFA from adipose tissue Pyridyl carbinol is one of these and when given to man this com pound lowers FFA levels (25) Ronicol Retard® a slow release preparation of pyridyl carbinol (24) has been reported to lower elevated plasma lipid levels in man (25 29 30) It appeared as if the dose of pyridyl carbinol needed to lower the plasma lipoprotein concentration was lower in this slow release preparation than that usually needed with plain nicotinic acid With regard to the above mentioned effect of nicotinic acid and pyridyl carbinol on plasma FFA we considered it of in

Table I *Sex age height weight plasma lipids blood sugar intravenous glucose tolerance (k value) and plasma FFA in the 14 patients receiving Ronicol Retard®*

Case	Sex	Age (y)	Height (cm)	Weight (kg)	Cholesterol (mg/100 ml)	Phospholipids (mg/100 ml)	Triglycerides (mmol/l)	Fasting blood sugar (mg/100 ml)	λ value	FFA[a] (mmol/l) 0 45 g	1 05 g
A Ja	♀	72	157	47	284	330	0 76	82	1 39	0 39	—
G Z	♀	61	165	69	247	284	0 73	78	—	0 45	—
H J	♂	62	178	73	247	304	1 45	98	—	0 88	—
S G	♀	55	159	68	326	405	2 01	93	1 37	0 33	0 19
G S	♀	55	164	75	332	396	2 41	80	1 13	0 72	1 16
J J	♂	55	171	90	194	262	1 57	93	1 05	0 38	0 48
M K	♀	65	164	68	266	323	3 07	77	1 98	0 34	0 19
Å J	♂	54	181	94	365	398	2 66	74	1 28	0 32	0 58
B S	♂	46	168	75	308	317	2 78	77	1 35	0 89	0 50
S B	♂	58	177	99	213	283	1 61	91	0 67	0 73	0 70
A K	♀	67	155	61	468	466	1 84	76	0 87	0 40	0 63
A Jo	♂	50	170	75	246	316	2 51	121	0 68	—	0 37
K K	♂	42	178	89	425	545	8 80	89	1 14	—	0 75
A A	♂	48	175	81	501	513	2 04	79	0 85	—	0 57
Range		42–72	155–178	47–99	194–501	262–545	0 73–8 80	74–121	0 68–1 39	0 32–0 89	0 19 1 16
Range previous study (17)		44–72	161–182	57 5–86 0	191–591	235–496	1 59–9 97	61–106	0 47–1 73	0 37–0 98[b]	0 31–1 12[c]

[a] The FFA values were taken at the beginning of the test
[b] Range for placebo test
[c] Range for nicotinic acid test

terest to compare the acute effects of Ronicol Retard® and of nicotinic acid on plasma levels of FFA and nicotinic acid after a single dose of the two preparations The technique used for this pose was identical to one we developed previ ly to study the effects of nicotinic acid and ~ebo (17)

The effects of two doses of Ronicol Retard® were studied 0 45 g and 1 05 g The first dose was given as this is the therapeutic single dose used in previous long term studies (30) The effect of the other dose 1 05 g was studied for comparison with 1 g of nicotinic acid which is the usual therapeutic single dose for nicotinic acid in treatment of hyperlipoproteinemia

To evaluate whether pyridyl carbinol in itself inhibited the release of FFA from adipose tissue or whether it had first to be metabolized to nicotinic acid we also studied the effect of this compound on lipolysis in adipose tissue in vitro

METHODS

The studies were done on hospitalized patients (Table I) Except H J and Å J who had benign elevations of blood pressure with diastolic levels around 110 mm of Hg (Table I) the only disease the patients had was ischemic cardiovascular disease either as a definite myocardial infarction (characteristic ECG and transaminase changes) at least six months before the study or as typical angina pectoris with ECG changes during exercise suggesting myocardial ischemia or intermittent claudication with oscillometric recordings typical of obliterating arterial disease None had any complicating disease all were in good nutritional condition and the only symptoms present in some were ischemic pains on effort No clinical diabetes (glycosuria acidosis vascular changes in eye grounds) was present, although one of them A Jo (Table I) had elevated fasting blood sugar (repeated determinations) but without increased plasma FFA concentration. Some of the patients had a lowered intravenous glucose tolerance in response to an intravenous glucose load Most of them had moderate hyperlipoproteinemia (hypertriglyceridemia and/or hypercholesterolemia) All patients had been followed by us at the outpatient department for at least several months many for years During this time they had had repeated blood samples withdrawn for lipid analysis which showed that the lipid levels were fairly stable The lipid levels showed in no case at the time of the study any major deviation from the patient's usual level No patient was on any special diet at the time of the study and in the hospital they had the ordinary hospital diet Nor was any patient on drugs known to affect lipid metabolism or on antihypertensive drugs. They had not had nicotinic acid before Table I shows that the patients given Ronicol Retard® were quite comparable to those previously given placebo and nicotinic acid under otherwise identical conditions (17)

Table II *Plasma levels of FFA (mmol/l) before and changes after Ronicol Retard® nicotinic acid and placebo*

Mean values ± standard errors of the mean are given The changes are calculated on the individual changes

Preparation	Before	Time (hours) after administration of drug ½	1	1½	2	3	4	5	6	7
Ronicol Retard® $n = 11$										
0 45 g	0 56 ±0 09	−0 29 ±0 08	−0 29 ±0 06	* −0 28 ±0 08	−0 14 ±0 13	0 32 ±0 16	0 55 ±0 17	0 41 ±0 09	0 39 ±0 11	
1 05 g	0 52 ±0 07	−0 18 ±0 05	−0 29 ±0 05	* −0 32 ±0 05	−0 29 ±0 05	−0 18 ±0 11	0 12 ±0 16	0 36 ±0 16	0 59 ±0 04	0 39 ±0 03 [a]
Nicotinic acid $n = 11$[b]										
1 g	0 53 ±0 03	−0 22 ±0 02	−0 31 ±0 02	−0 33 ±0 03	* −0 31 ±0 03	−0 22 ±0 04	* 0 76 ±0 16	* 1 18 ±0 14	1 00 ±0 16	
Placebo $n = 11$[b]										
1 g	0 59 ±0 03	−0 15 ±0 04	−0 17 ±0 07	−0 10 ±0 08	0 01 ±0 07	0 12 ±0 06	0 17 ±0 07	0 19 ±0 06	0 19* ±0 07	

and indicate statistical significance $P < 0.05$ < 0.01 and < 0.001 respectively for the changes from the basal level

[a] $n = 3$ [b] Data from (17)

The patients were carefully instructed about the purpose nature and possible side effects of the study The experimental procedure was strictly standardized and has been described in detail earlier (17) In the morning after fasting overnight a teflon catheter was inserted into an antecubital vein. A slow infusion of saline 0 9% kept the catheter patent and no heparin was injected The patients who stayed in bed throughout the study were given a light breakfast, consisting of one cup of tea and two slices of bread without butter The main purpose of the light breakfast was to reduce possible gastro intestinal discomfort caused by the nicotinic acid preparations About 45–60 min after the breakfast the first blood sample was withdrawn and the tablets were given by mouth Blood samples about 10 ml each time were then drawn into heparinized syringes and in most instances without stasis If stasis was necessary it was then used throughout the study The blood was immediately processed for determinations of plasma FFA and nicotinic acid.

Fourteen patients received Ronicol Retard® Six of them received either a dose of 0 45 g or 1 05 g and the remaining eight subjects received both doses on different occasions, 2 to 3 days apart. Plasma FFA were determined according to Dole (0) as modified by Trout et al (?7) The concentration of free nicotinic acid in plasma was determined as described previously from this laboratory (6) With this procedure pyridyl carbinol present in blood plasma gives about 10 of the optical density value of the same amount of nicotinic acid In 21 samples from nine patients we separated nicotinic acid and pyridyl carbinol from each other by thin layer chromatography in a system with n propanol/10% ammonium hydroxide (95 5) and subsequent eluation and determination of these two compounds (6) The R_f value of nicotinic acid was 0 2 and of pyridyl carbinol 0 6 in this system The statistical calculations were done as recommended by Snedecor (26)

For the in vitro experiments epididymal adipose tissue pieces were obtained from fed Sprague Dawley rats (180–200 g) and incubated in vitro as described in detail elsewhere (3 5) The pieces were randomized between incubation flasks containing Krebs Ringer bicarbonat buffer 2% human serum albumin (Kabi Stockholm Sweden) and 0 1 glucose Lipolysis was determined by enzymatic measurement of the amount of glycerol (28) released into the medium during incubation for 1 h at 37 C.

RESULTS

Plasma FFA

There were no significant differences in the mean plasma levels of FFA before administration of the two doses of Ronicol Retard nicotinic acid or placebo (Table II) Already 30 min after administration of *0 45 g Ronicol Retard®* the FFA had decreased from a mean concentration of 0 56 to 0 27 mmole/l (Figs 1 and 2 Table II) At about 1½ to 2 h the FFA concentration started to increase (Table II) and reached a maximal level on the average 0 55 mmole/l above the initial concentration 4 h after the administration of Ronicol Retard®

With *1 05 g of Ronicol Retard®* the FFA concentration was depressed during 2–3 h (Figs 3 and 4 Table II) The FFA concentration then increased to a maximal level 0 59 mmole/l above

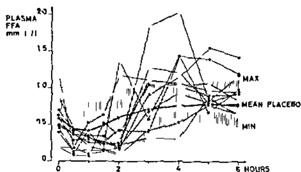


Fig 1 Comparison of individual values for plasma FFA levels after 0 45 g of Ronicol Retard® taken by mouth at 0 hours with mean value and range (max and min values) for values after 1 g of placebo by mouth (17)

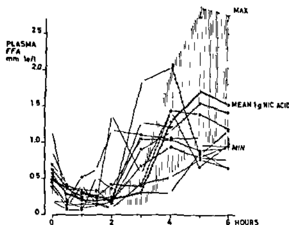


Fig 2 Comparison of individual values for plasma FFA levels after 0 45 g of Ronicol Retard® taken by mouth at 0 hours with mean value and range (max and min values) after 1 g of nicotinic acid by mouth (17)

the initial level at about 6 h after the administration of the drug

Nicotinic acid 1 g caused a significant depression of the plasma FFA concentration for about 3 h (Table II) There was then a pronounced overshoot up to maximal level of 1 71 mmole/l at about 5 h after the administration of nicotinic acid

When *placebo* was given the concentration of FFA fell at 30 and 60 min (Table II) presumably at least partly as a result of the light breakfast From one hour on the FFA level rose slowly and remained fairly stable during the last two hours

The individual FFA values after *0 45 g of Ronicol Retard®* are compared to the mean and range of values obtained after placebo in Fig 1 and after nicotinic acid in Fig 2 As seen up to about two hours nine of the eleven patients given *0 45 g were below the average placebo value* After 3 h most patients were above the mean placebo level and four patients at 3 h and seven at 4 h were above the maximal FFA level seen after placebo When compared to values found after 1 g of nicotinic acid (Fig 2) it is clear that the effect of 0 45 Ronicol Retard® on plasma FFA had a lesser duration in all but two patients For instance at 2 and 3 h four and seven of the patients had FFA levels above maximal FFA values after nicotinic acid at corresponding times

The individual FFA levels after *1 05 g of Ronicol Retard®* are compared to mean and range for levels seen after placebo (Fig 3) and nicotinic acid (Fig 4) Fig 3 shows that in all patients up to 2 h in ten up to 3 h and in seven up to 4 h

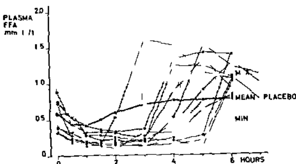


Fig 3 Comparison of individual values for plasma FFA levels after 1 05 g of Ronicol Retard® taken by mouth at 0 hours with mean value and range (max and min values) after 1 g of placebo by mouth (1)

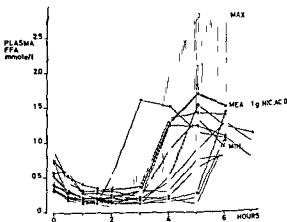


Fig 4 Comparison of individual values for plasma FFA levels after 1 05 g of Ronicol Retard® taken by mouth at 0 hours with mean value and range (max and min values) after 1 g of nicotinic acid by mouth (17)

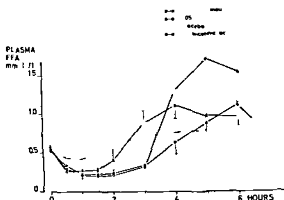


Fig 5 Comparison of mean levels of plasma FFA in patients after the two doses of Ronicol Retard® and of placebo and nicotinic acid Bars indicate standard error of mean x—x 0 45 g by mouth ●—● 1 05 g ——— placebo O—O 1 g nicotinic acid

the concentration of FFA in plasma was lower than the mean level after placebo At 4 5 and 6 h two five and four patients respectively showed an overshoot above placebo values When the results are compared to the effect of 1 g of nicotinic acid Fig 4 demonstrates that with the exception of one case the FFA levels in the patients given Ronicol Retard® 1 05 g followed the nicotinic acid pattern up to three hours Thereafter however they in general tended to be lower It is especially noteworthy that the pronounced rebound effect seen after 1 g of nicotinic acid does not appear to the same extent after 1 05 g of Ronicol Retard® at least not within 6 to 7 h after administration At 5 as well as 6 h all FFA values were below the mean value for 1 g of nicotinic acid

The group mean levels of FFA after the two doses of Ronicol Retard® after nicotinic acid and after placebo are shown in Fig 5 The larger dose of Ronicol Retard® as compared to the lower gave the same FFA level up to about 2 h but at 3 and 4 h the levels were significantly lower Fig 5 also clearly depicts the overshoot in FFA levels after 1 g of nicotinic acid and the almost complete absence of this phenomenon after 1 05 g of Ronicol Retard® at least up to 6–7 h

Free nicotinic acid in blood plasma

The results of the 21 analyses of nicotinic acid and pyridyl carbinol by means of thin layer chromatography are given in Table III It can be seen that in all instances early after administration

Table III *Plasma levels of free nicotinic acid (NA) determined directly and after separation by thin layer chromatography (TLC) and plasma levels of pyridyl carbinol (PC) determined after separation by TLC*

Plasma was taken at various times after 1 05 g of Ronicol Retard® by mouth

Case	Time (h)	NA Direct determination (µg/ml)	NA Determined after TLC (µg/ml)	PC Determined after TLC (µg/ml)
K K	1½	5 4	5 5	2 6
K K	4	1 5	1 3	3 9
B S	1½	1 2	0 9	8 5
B S	3	3 9	2 5	3 7
A Jo	2	14 8	13 2	4 3
Å J	1½	1 3	1 0	5 5
Å J	3	4 8	4 3	7 7
J J	1½	3 4	3 2	5 5
J J	3	4 8	4 1	10 0
S G	1½	4 5	3 8	7 9
M K	½	1 9	1 7	0 7
M K	1½	6 0	5 7	8 0
M K	3	4 7	4 8	2 3
M K	4	0 9	0 6	3 7
A K	1	0 4	0	0
A K	1½	2 4	2 0	2 3
A K	3	6 7	9 6	4 0
A K	5	1 3	1 7	1 7
A A	1½	2 5	1 9	0
A A	3	4 1	3 2	0
A A	5	0 4	0 5	0

(1/ h) as well as late (5 h) there is a good agreement with the direct analysis of nicotinic acid which includes the interference of pyridyl carbinol and the values obtained after separation of nicotinic acid from pyridyl carbinol on thin layer chromatography plates The direct analyses give only slightly higher values even in the presence of considerable amounts of pyridyl carbinol The explanation of this is that the interference of pyridyl carbinol is rather low as this compound only gives about one tenth as much color as nicotinic acid when analyzed in the same amounts

With *0 45 g of Ronicol Retard®* (Tables IV and VI) there was a slow increase in the plasma concentration of free nicotinic acid The maximal mean level of 1 1 µg/ml was reached after 2 h and the concentration was usually zero or almost zero after 6 h

With *1 05 g of Ronicol Retard®* (Tables V and VI) the maximal mean concentration of 5 2 µg/ml was reached after 3 h

After administration of *1 g of nicotinic acid* on the other hand there was a rapid and marked

Table IV *Plasma levels of FFA (mmol/l) and free nicotinic acid (µg/ml) determined directly after 0 45 g of Ronicol Retard® by mouth at 0 hours*

Case	Time (h)	0	½	1	1½	2	3	4	5	6
A Ja	Nic	0	0	0 3	1 0	0 5	0 1	0 1	0	0
	FFA	0 39	0 11	0 41	0 16	0 11	1 85	2 04	0 84	1 17
G Z	Nic	0	0	1 3	0 9	0 1	0 5	0 2	0	0
	FFA	0 45	0 14	0 15	0 14	1 38	0 58	1 44	1 40	1 19
H J	Nic	0	0 2	0 2	0 4	1 5	0 5	0 3	0 2	0 2
	FFA	0 88	0 41	0 35	0 34	0 16	0 91	1 15	1 55	1 42
S G	Nic	0	0 3	1 2	1 4	0 9	1 2	0 3	0 6	0 6
	FFA	0 19	0 18	0 10	0 13	0 19	0 68	0 94	0 80	0 76
G S	Nic	0	1 1	0 8	0 7	0 5	0 4	0 6	0 5	0 1
	FFA	1 16	0 19	0 52	0 63	1 17	1 11	1 05	1 07	0 83
J J	Nic	0 1	0 3	0 4	1 3	1 5	0 5	0 4	0 3	0 3
	FFA	0 48	0 37	0 34	0 23	0 25	1 10	1 07	0 97	0 95
M K	Nic	0	0	1 1	0 5	1 7	0 4	0	0	0
	FFA	0 19	0 08	0 07	0 52	0 37	1 05	1 04	0 90	0 92
Å J	Nic	0	0	0 1	0 3	0 1	0 1	0 5	0 5	0 4
	FFA	0 58	0 29	0 29	0 76	0 22	0 32	0 30	0 87	0 64
B S	Nic	0	0 1	0 1	0 1	0 3	0 2	0 6	0 4	0
	FFA	0 50	0 37	0 25	0 25	0 43	0 40	1 43	0 67	0 98
S B	Nic	0 2	0	0	0 9	0 8	0 6	0 2	0 2	0 2
	FFA	0 70	0 42	0 32	0 26	0 19	1 28	1 21	0 81	0 94
A K	Nic	0 1	0	0 1	0 3	3 8	0 4	0 4	0	0 2
	FFA	0 63	0 39	0 20	0 15	0 09	0 43	0 52	0 77	0 65

increase in plasma concentration of nicotinic acid The maximal level around 25–30 µg/ml was seen already after 30–60 min The nicotinic acid concentration then progressively decreased and reached [illegible]o levels at 6 h

Relationship between concentrations of FFA and free nicotinic acid in blood plasma (Tables IV V VI and VII)

Already 30 min after *0 45 g of Ronicol Retard®* the FFA concentration had decreased although

Table V *Plasma levels of FFA (mmol/l) and free nicotinic acid (µg/ml) determined directly after 1 05 g of Ronicol Retard® by mouth at 0 hours*

Case	Time (h)	0	½	1	1½	2	3	4	5	6	7
S G	Nic	0	0	0 4	4 5	1 8	11 3	4 6	4 5	1 4	
	FFA	0 33	0 22	0 18	0 17	0 17	0 13	0 20	0 12	1 03	
G S	Ni	0 2	2 2	2 2	4 2	1 5	0 8	0 6	0 1	0 2	
	FFA	0 72	0 23	0 19	0 15	0 53	1 62	1 53	1 16	1 07	
J J	Nic	0 2	0 2	0 2	3 4	3 4	4 8	1 0	0 7	0 6	
	FFA	0 38	0 32	0 [illegible]0	0 21	0 21	0 18	0 23	0 65	0 84	
M K	Nic	0 1	1 9	2 6	6 0	6 9	4 7	0 9	0 4	0 1	
	FFA	0 34	0 18	0 13	0 15	0 16	0 21	1 25	1 25	1 10	
Å J	Nic	0 1	0 1	0	1 3	1 0	4 8	2 0	0 7	0 4	
	FFA	0 32	0 21	0 16	0 13	0 09	0 32	0 31	0 77	0 81	
B S	Nic	0	0 1	0	1 2	1 0	3 9	1 9	3 6	1 5	
	FFA	0 89	0 43	0 32	0 32	0 31	0 23	0 19	0 28	1 45	
S B	Nic	0	0	0 2	0 1	5 7	7 9	1 1	0 4	0	0 1
	FFA	0 73	0 54	0 28	0 20	0 22	0 13	0 51	1 55	1 24	1 13
A K	Nic	0 1	0 6	0 4	2 4	3 2	6 7	2 1	1 3	0 4	0 7
	FFA	0 40	0 28	0 18	0 13	0 14	0 13	0 15	0 [illegible]1	1 09	0 76
A Jo	Nic	0	0	5 8	9 2	14 8	2 9	1 3	1 0	0 4	
	FFA	0 32	0 24	0 18	0 19	0 18	0 22	0 90	1 35	0 92	
K K	Nic	0 7	0 6	2 0	5 4	5 4	5 9	1 5	1 0	0 9	
	FFA	0 75	0 52	0 41	0 35	0 33	0 37	1 35	1 44	1 41	
A A	Nic	0	0	0 2	2 5	3 3	4 1	0 5	0 4	0 4	0 5
	FFA	0 57	0 60	0 28	0 28	0 25	0 20	0 44	0 92	1 30	1.00

Table VI *Plasma levels of free nicotinic acid (µg/ml) after Ronicol Retard*[a] *and nicotinic acid by mouth at 0 hours*

		0	½	1	1½	2	3	4	5	6	
Ronicol Retard[a] $n = 11$											
0 45 g	Mean	0	0 2	0 5	0 7	1 1	0 4	0 3	0 3	0 2	
	Range	0–0 2	0 1 1	0 1–1 3	0 1–1 4	0 1–3 8	0 1–1 2	0 1–0 6	0–0 6	0–0 6	
Ronicol Retard[a] $n = 11$											
1 05 g	Mean	0 1	0 5	1 3	3 6	4 4	5 2	1 6	1 3	0 6	0 4 ($n = 3$)
	Range	0–0 7	0– 2	0–5 8	0 1–9 2	1 0–14 8	0 8–11 3	0 6–4 6	0 1–4 5	0 1 1 5	0 1–0 5
Nicotinic acid $n = 9$											
1 g		0 1	26 1	27 6	23 7	14 8	3 8	1 0	0 4	0 2	
		0–0 4	9 4–38 5	20 9–34 0	16 3–32 1	10 0–22 6	1 4–9 7	0 2–2 6	0–0 9	0–0 4	

[a] Data from (17)

there was still no measurable increase in free nicotinic acid concentration in the blood of several of the patients This initial decrease may at least partly be explained by the light breakfast as there was a small initial fall in FFA also in the placebo group

With *0 45 g of Ronicol Retard*® the FFA levels remained depressed during 1–4 h and the nicotinic acid concentration varied between 0 1–3 8 µg/ml during that time The plasma FFA concentration started to increase when the concentration of nicotinic acid in plasma fell from a mean level of 1 3 to 0 5 µg/ml (Table VII)

After the higher dose of *Ronicol Retard*® *1 05 g* the plasma FFA levels were depressed during 1½ to 6 h (mean 2–3 h) and the maximal nicotinic acid concentration in blood was seen after about 2–3 h There were pronounced variations in the concentration of nicotinic acid in plasma between the different patients Depressed FFA levels in the blood were seen with a nicotinic acid concentration varying from 0 1 to 14 8 µg/ml Table VII shows that the FFA concentration started to rise when the nicotinic acid concentration fell from a mean level of 3 8 to 1 1 µg/ml

After *1 g of nicotinic acid* however the con

Table VII *Concentration of FFA and free nicotinic in blood plasma when the plasma levels of FFA started to rise after the initial depression in FFA concentration after Ronicol Retard*[a] *and nicotinic acid*

This rise was assumed to start when the FFA concentration increased 0 1 mmol/l or more from one hour to the next and the figures give the values at the start of the rise and the values one hour later

Time		FFA mmol/l		Nicotinic acid µg/ml	
		Start of rise	One hour later	Start of rise	One hour later
Ronicol Retard[a] $n = 11$					
0 45 g	Mean	0 20	0 95	1 3	0 5
	Range	0 09–0 37	0 43–1 85	0 1–3 8	0 1 1 2
Ronicol Retard[a] $n = 11$					
0 45 g	Mean	0 22	0 91	3 8	1 1
	Range	0 12–0 37	0 44–1 45	1 0–7 9	1 0–7 9
Nicotinic acid $n = 9$					
1 g	Mean	0_6	0 96	8 8	2.7
	Range	0 10–0 32	0 37–1 93	1 4–26 0	0 1 13 0

[a] Data from (17)

Table VIII *Symptoms after administration of Ronicol Retard® 0 45 and 1 05 g by mouth at zero hours*

Time (h)			0	½	1	1½	2	3	4	5	6	7
A Ja	0 45 g											
G Z	0 45 g				( )							
H J	0 45 g				(a)			C				
S G	0 45 g											
		1 05 g			(c)	(b)	(a)	C				
G S	0 45 g			(b)								
		1 05 g		(c)		(a)						
J I	0 45 g											
		1 05 g				( )	(c)		C			
M K	0 45 g		H	H	H(a)	H	HC	H	H	H	H	H
		1 05 g		(c)	(c)	(b)	( )					
Å J	0 45 g					( )	(b)					
		1 05 g				( )						
B S	0 45 g					( )	C	C	C	C	C	C
		1 05 g				(b)	( )	C	C	C		
S B	0 45 g											
		1 05 g					(b)	( )				
A K	0 45 g						D( )					
		1 05 g				D(c)	(b)					
A Jo	—											
		1 05 g				(c)	(b)					
K K	—											
		1 05 g			(b)	( )				H	H	H
A A	—											
		1 05 g				(b)	(a)	( )				

[a] Slight flush in the face
[b] Marked flush in the face neck and upper part of the body
[c] Marked flush in the face and the whole body

D = dizzy C = chilly H = headache

centration of FFA in plasma began to increase at higher plasma levels of nicotinic acid in general as shown in Table VII when the nicotinic acid concentration fell from a mean level of 8 8 to 2 7 μg/ml

Symptoms (Table VIII)

Ronicol Retard® in a dose of 0 45 g produced a slight flush in 7 of 11 patients 30 min to 2 h after administration With the higher dose 1 05 g a flush was seen in all subjects and it was more intensive and of longer duration

With 1 g of nicotinic acid too there was always a marked flush for details see Carlson et al (17)

In vitro studies

The effect on lipolysis of the addition of nicotinic acid and of pyridyl carbinol to adipose tissue in vitro is shown in Table IX Nicotinic acid at a concentration of 0 1 μg/ml incubation medium significantly depressed the release of glycerol by more than 50% from the incubated tissues as demonstrated earlier (4) Pyridyl carbinol however in concentrations of 0 1 and 1 μg per ml medium had no discernible effect on lipolysis When the concentration was raised to 10 μg per ml medium pyridyl carbinol exerted a slight inhibition

Table IX *A typical result obtained when adipose tissue is incubated in vitro with nicotinic acid and pyridyl carbinol*

Mean value (8 incubation flasks) ± S E M for the release of glycerol (μmol/g/h) is given

Addition to the medium		Nicotinic acid	Pyridyl carbinol		
	0	0 1 μg/ml	0 1 μg/ml	1 μg/ml	10 μg/ml
	1 44 ±0 08	0 69 ±0 08	1 47 ±0 07	1 46 ±0 05	1 14 ±0 05

DISCUSSION

The data on the levels of free nicotinic acid in blood plasma after taking Ronicol Retard® or nicotinic acid showed quite different patterns Plain nicotinic acid rapidly resulted in high peak levels soon levelling off Ronicol Retard® on the other hand did not give early peaks and the blood concentration curve remained fairly flat This difference might be ascribed to two factors First the pharmaceutical preparation delays the resorption from the gastro-intestinal tract (24) Secondly pyridyl carbinol has to be metabolized in the body to be oxidized to nicotinic acid This process however apparently occurs at a rapid rate in the liver (24) This suggests that the pharmaceutical preparation is the major factor responsible for the flat plasma concentration of the free nicotinic acid curve after Ronicol Retard®

Several studies including investigations of excess mobilization and inhibition (5 7 14) of FFA mobilization indicate that the flux of FFA from adipose tissue to the liver is an important factor in regulating the concentration of triglycerides in the liver as well as in plasma (10 14 15 16) It has therefore been proposed that the inhibitory effect of nicotinic acid on FFA mobilization at least partly explains why nicotinic acid lowers the plasma lipoprotein concentration (4 10 11 16)

It was reported that Ronicol Retard® in doses smaller than those usually needed with nicotinic acid effectively lowered the plasma cholesterol concentration in long term studies (30) If the cholesterol lowering effect of both nicotinic acid and Ronicol Retard® are related to lowering of FFA one might expect that Ronicol Retard® should induce a more long lasting decrease of the plasma FFA concentration than a similar dose of nicotinic acid However from the present study it is evident that the duration of the lowering of FFA below placebo levels was almost the same after 1 05 g of Ronicol Retard® and after 1 g nicotinic acid but *about one hour less with 0 45 g* Ronicol Retard® This latter dose is the dose which has been reported when given three times daily to lower cholesterol in a way similar to or even better than 1 g of nicotinic acid three times daily If we accept that 0 45 g of Ronicol Retard® has at least a similar effect on plasma cholesterol levels to that of 1 g of nicotinic acid keeping in mind that *no direct comparisons* have been made it seems difficult to reconcile the lowering effects of these two compounds on plasma FFA levels with their effect on the concentration of cholesterol in plasma This suggests that effects other than inhibition of FFA mobilization may be related to the cholesterol lowering effect of nicotinic acid and/or pyridyl carbinol

However nicotinic acid not only induces a fall in plasma FFA as the fall in FFA concentration after a single dose of nicotinic acid is always followed by a FFA rise above the initial level an overshoot (11 17 23) It appears likely that during this overshoot period there is an increased FFA mobilization which might stimulate the hepatic production of lipoproteins thus counteracting the lipid lowering effect of nicotinic acid As a matter of fact the overshoot of FFA levels occurring when the effect of nicotinic acid on lipolysis vanishes (11) has raised the question whether chronic treatment with nicotinic acid indeed lowers the turnover of FFA integrated over the 24 h period (17 19 21 22) It may be quite relevant in the context of lowering of plasma cholesterol that we could demonstrate here that the FFA overshoot was less pronounced after Ronicol Retard® than after nicotinic acid It is possible that this finding explains why Ronicol Retard® may be more effective than nicotinic acid in lowering the plasma lipoprotein concentration during long term treatment Furthermore it should be stressed that one certainly cannot extrapolate from these studies performed under strictly standardized conditions and made as quiet and pleasant as possible for the patient to daily life situations

The active substance in Ronicol Retard® pyridyl carbinol in contrast to nicotinic acid produced no inhibition of lipolysis in adipose tissue in vitro at concentrations of 0 1 and 1 μg/ml The slight effect obtained at concentrations of 10 μg/ml could be explained if the preparation used contained less than one per cent of nicotinic acid as impurity This lack of direct action on adipose tissues strongly suggests that Ronicol Retard® influences FFA mobilization after it has been metabolized to nicotinic acid One can also conclude from the in vitro data that the levels of pyridyl carbinol in plasma very likely had no major influence on the FFA levels

There were no correlations between the concentrations of FFA and nicotinic acid in the blood This was most obvious at the time for the start of

the FFA overshoot With 0 45 g Ronicol Retard® the mean nicotinic acid concentration decreased from 1 3 to 0 5 μg/ml with 1 05 g Ronicol Retard® from 3 8 to 1 1 and with nicotinic acid from 8 8 to 2 7 μg/ml when the mean FFA concentration increased in all three groups from around 0 2 to 0 9 mmole/l In fact in one of the patients given nicotinic acid the FFA concentration increased although the concentration of free nicotinic acid in the blood still was at a level of 13 μg/ml This indicates that the concentration of free nicotinic acid in the blood gives little or no information about the inhibitory effect of a nicotinic acid preparation on FFA mobilization It is probable that determinations of nicotinic acid within adipose tissue would be more valuable in this respect Also the difference in overshoot between nicotinic acid and pyridyl carbinol is noteworthy Our results strongly suggest that it is not the inhibition of lipolysis per se that induces the overshoot This result is compatible with the in vitro results obtained by Carlson and Micheli (9)

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DESTRUCTION OF ERYTHROCYTES DURING EXPERIMENTAL FEVER

Quantitative Aspects

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Abstract A simple analytical model has been worked out for quantitative assessment of temporary shifts in erythrocyte survival curves after labelling with radioactive tracers This model was applied to ^{51}Cr and DF^{32}P curves from rabbits exposed to experimental fever induced by either bacterial pyrogen heated milk or external heating in a climatic chamber Thereby a quantitative expression of the fever induced erythrocyte destruction was obtained

In all three methods for elevating the body temperature experimentally the studies showed an increased loss of activity during the fever either in terms of the slope of the curve segment concerned with fever compared to the pre or post febrile periods or in terms of the deviation of the observed curves from the expected, spontaneous course in the rabbit The maximum loss occurred soon after the initiation of fever and prolongation of the fever period did not cause a continued increase in the destruction of the labelled erythrocytes A certain correlation was found between the initial action of heat and the magnitude of the erythrocyte destruction

After the cessation of a short lasting period of fever the tracer curve again approached the expected spontaneous course explained by an age-dependent variation in the heat susceptibility of the erythrocytes. By using the age-distribution function for rabbit erythrocytes an explanation of the demonstrated differences in the simultaneous changes in radioactivity and haemoglobin or PCV is attempted

It is concluded that elevated body temperature shortens the mean survival time of the red cells The average reduction of the red cell mass in the present study was about 15% and the maximum loss about 35%

Haemolysis is a contributory cause of anaemia in many diseases in which fever is a predominant clinical feature In erythrocyte survival studies in rabbits using autologous erythrocytes labelled with Na ^{51}CrO$_4$ or diisopropylfluorophosphate (DF^{32}P) an increased destruction of red cells has been demonstrated during experimental fever (5) These investigations are continued by the present study in which an attempt has been made to analyse the fever induced haemolysis quantitatively

[1] Study performed during the tenure of a Research Fellowship from the Danish Anti-cancer League

MATERIAL AND METHODS

Rabbits of both sexes weighing 2500–4000 g were used Labelling of red cells was done by Na ^{51}CrO and DF^{32}P The experimental methods used to induce fever were 1 i.v injection of bacterial pyrogen 2 i.m injection of heated milk and 3 external heating in a climatic chamber For labelling procedure sampling and radioactive measurements standard haematological studies and recording of temperature (Electro Universal Thermometer lent by the Control Laboratories of The Danish Pharmacist Association) compare the previous publication (5) The fever response was expressed as a Fever Index using the area below the temperature curve (Δ Degree C × hours) and was determined by weighing the cut-out area.

Model in the quantitative analysis of fever induced erythrocyte destruction

The experimental results are illustrated in Fig 1 The spontaneous course of the erythrocyte survival curve was observed during 10 days after Na$_2$ CrO labelling (10 days after DF^{32}P labelling) A period of fever was then introduced for 5–6 days with daily elevations of temperature lasting 10–14 hours (in induced hyperthermia only 6–8 hours) Fig 1 demonstrates that there is a fall in the haemoglobin level and simultaneously a steeper drop of the survival curve (relative activity per ml blood) in connection with the fever Quite rapidly reticulocytosis appears and after the fever has ceased the haemoglobin level returns to normal and the tracer curve again flattens out

Normal erythrocyte survival in rabbits labelled with Na$_2$^{51}CrO or DF^{32}P will appear curvilinear in a semilogarithmic coordinate system with a slightly downward tendency If this curve is divided into smaller time units, each of these units will to all practical purposes, present itself as a straight line Accordingly the erythrocyte sur

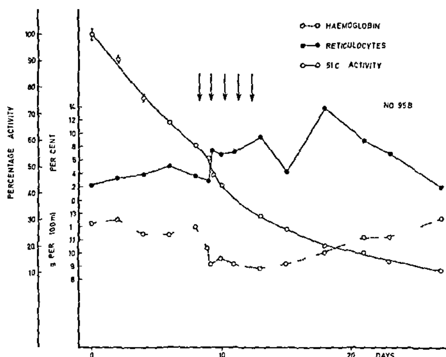


Fig 1 The influence of five days of pyrogen induced fever on the survival of ⁵¹Cr label led erythrocytes haemoglobin and reticulocyte values in a rabbit. Activity expressed as counts per ml blood in per centage of value at time zero

vival curves in the present material were divided into an initial pre febrile segment (I) a febrile segment (F) and one or two post febrile segments (P_1 and P) The equa ns for the relevant segments for each rabbit were deter ed by the method of least squares after log trans rmation of the value of radioactivity per ml blood. A imilar division was made in the normal group and a comparison was performed by means of the slopes of the curve segments To obtain a clearer expression of the induced loss of erythrocytes the initial curve segment which is the only expression of the spontaneous course in the fever exposed animals was extrapolated for all the rabbits through the subsequent time units Comparison between the difference from this extrapolated line in the normal rabbits and in the fever-exposed rabbits will give a description of the fever induced haemolysis The per cental loss of activity as a result of fever was calculated for each rabbit at different times according to the equa tion

$$\frac{\left[F_{hyp}\ \frac{C_{b}}{C_{hyp}}\right] - F_{bs}}{F_{hyp}\ \frac{C_{bs}}{C_{hyp}}}\ 100$$

(F_{yp} = calculated activity on extrapolated line from fever exposed animals F_b = observed activity from fever-ex posed animals C_b and C_b = corresponding mean values for the control group)

Table I *Slope of curve segments in ⁵¹Cr studies in rabbits before during and after experimental fever*

Method of fever	No of animals	Periods a_I (day 1–10)	a_F (day 10–16)	a_P (day 16–25)	a_P (day 25–35)	$\frac{a_F}{a_I}$	$\frac{a_P}{a_I}$	$\frac{a_P}{a_I}$
Controls	5	−0 0273 ±0 0040	−0 0271 ±0 0041	−0 0[illegible]59 ±0 0043	−0 0297 ±0 0056	1 [illegible]3 ±0 07	1 17 ±0 16	1.34 ±0 21
Pyrogen	16	−0 0[illegible]03 ±0 0036	−0 0397 ±0 0161	−0 0208 ±0 0072	−0 0[illegible]49 ±0 0070	1 94 ±0 62	1 03 ±0 28	1 25 ±0 34
Heated milk	5	−0 0[illegible]00 ±0 0026	−0 0438 ±0 0090	−0 0[illegible]06 ±0 0077	−0 0[illegible]36 ±0 004[illegible]	2 21 ±0 46	1 05 ±0 43	1 19 ±0 23
External heating	4	−0 0158 ±0 0053	−0 0324 ±0 0119	−0 0205 ±0 0045	−0 0201 ±0 0082	2 25 ±0 98	1 36 ±0 [illegible]5	1 37 ±0 58
Mean of fever groups		−0 0195 ±0 0039	−0 0393 ±0 0144	−0 0[illegible]07 ±0 0067	−0 0[illegible]39 ±0 0067	2 04 ±0 65	1 08 ±0 32	1 [illegible]5 ±0 36
t test		$p>0\ 10$	$p<0\ 005$	$p>0\ 10$	$p>0\ 05$	$p<0\ 001$	$p>0\ 50$	$p>0\ 50$

Table II *Observed ^{51}Cr activity at various times after experimental fever in rabbits in relation to hypothetical values obtained from extrapolation of pre febrile curve segment*

Method of fever	No of animals	$t=16$ ($t_F=1$)	$t=21$ ($t_F=6$)	$t=25$ ($t_F=10$)	$t=30$ ($t_F=15$)
Controls	5	95 1±2.1	91 7±4 7	88 5±7 7	80 6±8 7
Pyrogen	16	82.4±10 7 ($p<0\ 001$)	82.4±12.5 ($p<0\ 025$)	83 1±16 4 ($p>0\ 40$)	77.3±19 6 ($p>0\ 70$)
Heated milk	5	78 1±7.2 ($p<0\ 005$)	77 7±11.3 ($p<0\ 05$)	78 4±16 9 ($p>0\ 25$)	70 8±26.2 ($p>0\ 40$)
External heating	4	81.3±11.2 ($p<0\ 05$)	76 8±11 8 ($p<0\ 05$)	74 3±12.0 ($p>0\ 05$)	72.1±10 7 ($p>0\ 20$)
Mean of fever groups		81 5±9 9	80 6±12.0	80 7±15 7	75 1±19 4
t-test		$p<0\ 001$	$p<0\ 005$	$p>0.25$	$p>0\ 50$

t = days after labelling t_F = days from end of fever period

RESULTS

I *Studies by the ^{51}Cr Method*

1 *Slope of curve segments*

The effect of a 5-day fever period upon the erythrocyte survival curve was studied on 25 rabbits starting 10 days after labelling with Na $^{51}CrO_4$ In 16 rabbits the fever was induced by bacterial pyrogen in five by heated milk, and in four by external heating The curves were fractionated into the following segments I = 1st–10th day F = 10th–16th day P_1 = 16th–25th day and P = 25th–35th day A normal group was divided in the same way Table I gives the slopes for the relevant segments in the respective groups as well as the mean value for the fever-exposed groups It is apparent that the fever period induces a significantly steeper course of the survival curve while the post febrile periods tend to show a smaller slope than the control group

Even more distinct is that part of Table I which sets out the slopes for the fever and post fever periods in relation to the slope for the first period which represents the spontaneous destruction pattern for the individual rabbit The relation between the 2nd and 1st period (a_F/a_I) is significantly higher in the fever groups while the corresponding value for the post febrile periods (a_P / a_I and a_P /a_I) does not differ significantly from the normal material

2 *Differences of observations from an extrapolation of the pre febrile curve segment*

Table II sets out the observed ^{51}Cr activities in the same material obtained by regression analysis of the individual curve segments The results are expressed in per cent of the hypothetical values from the extrapolated prolongation of the pre febrile segment calculated for the same time and starting immediately after the cessation of fever (16th day) From the results for the control group it is apparent that there is a tendency to a de

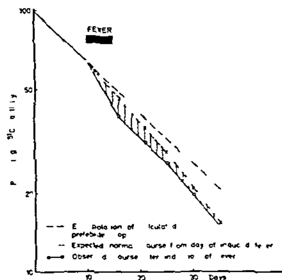


Fig Mean result from studies on the survival of ^{51}Cr labelled erythrocytes in 25 rabbits exposed to a five-day period of experimental fever produced by injection of bacterial pyrogen or heated milk or by external heating The fever period starts ten days after the labelling. The figure demonstrates the basis for the calculation of lost activity by showing extrapolation of pre febrile curve segment the expected normal course (calculated from the survival of red cells in normal rabbits) and the observed course (cf text)

Table III *Loss of ^{51}Cr activity haemoglobin and PCV (percentage) after five days experimental fever in rabbits*

Method of fever	Animal no	^{51}Cr	Haemoglobin	Haemoglobin–^{51}Cr	PCV	PCV–^{51}Cr	PCV–haemoglobin
Pyrogen	1B	22 3	17 6	−4 7	22 2	−0 1	4 6
	2B	10 9	10 3	−0 6	11 8	0 9	1 5
	3B	15 5	17 1	1 6	19 4	3 9	2.3
	5B	3 3	18 3	15 0	2[illegible] 2	18 9	3 9
	7B	47 0	34 8	−12 2	30 3	−16 7	−4 5
	8B	17 6	15 0	−2 6	16 7	−0 9	1 7
	9B	13 4	10 1	−3 3	6 2	−7 2	−3 9
	10B	0 3	4 4	4 1	8 5	8 2	4 1
	88A	16 5	19 7	3 2	20 6	4 1	0 9
	89A	0 6	8 1	7 5	11 4	10 8	3 3
	90A	6 1	15 1	9 0	18 2	12 1	3 1
	91A	9 1	8 1	−1 0	8 6	−0 5	0 5
	92A	5 6	13 7	8 1	17 5	11 9	3 8
	93A	8 8	13 0	4 2	14 3	5 5	1 3
	94A	16 4	31 7	15 3	41 7	25 3	10 0
	96A	17 1	19 7	2 6	21 6	4 5	1 9
Mean ± S D		13 16 ±11 09	16 04 ± 8 06	2 89 ±7 25	18 20 ±8 89	5 04 ±9 91	2 16 ±3 34
Heated milk	14B	14 5	19 1	4 6	17 1	2 6	−2 0
	23B	13 2	17 5	4 3	16 7	3 5	−0 8
	24B	11 2	15 0	3 8	12 1	0 9	−2.9
	25B	20 5	22 5	2 0	21 2	0 7	−1 3
	26B	29 9	30 0	0 1	34 3	4 4	4 3
Mean ± S D		17 86 ±7 55	20 82 ±5 74	2.96 ±1 89	20 28 ±8 48	2 42 ±1 61	−0 54 ±2 16
xternal eating	12B	4 2	15 3	11 1	8 3	4 1	−7 0
	30B	29 3			30 0	0 7	
	42B	5 9	13 3	7 4	8 1	2 2	−5 2
	13B	15 3			14 3	−1 0	
Mean ± S D		13 68 ±11 49	16 93 ±1 40	9 [illegible]5 ±2 54	15 17 ±10 [illegible]0	1 50 ±2 17	−6 1 ±1 23
Mean of fever groups		14 14 ±10 39	16 93 ±7 42	3 46 ±6 12	18 13 ±8 60	3 95 ±8 05	0 85 ±3 [illegible]0
t test				$p<0\ 02$		$p<0\ 025$	$p>0\ 40$

crease with time corresponding to the shape of the normal survival curve On the first day after cessation of fever the value of remaining activity is significantly lower in the fever exposed groups than in the control group In other words the fever has entailed an increased loss of activity The difference between the fever group and the control group decreases during the post febrile period when calculated for each day it is not significant after the 21st day These results are illustrated in Fig 2 which graphically shows the mean results for all rabbits of the fever group It shows the pre febrile curve segment with the extrapolated prolongation also the expected spon taneous course calculated on the basis of the deviation from the extrapolated line in the control group and lastly the observed course Again there is a distinctly lower value of the observations after cessation of fever the difference between expected and observed course then decreases gradually but a difference remains throughout the experimental period Table III lists the percental loss of ^{51}Cr caused by the fever calculated on the first day after its cessation For the entire material this loss amounts to about 14% of the residual activity

3 *Changes in the survival curves during the fever and significance of the duration of fever*

The changes in ^{51}Cr activity during the fever period itself were investigated by frequent samp lings and by extending the fever period for 10 days In order to avoid development of tolerance

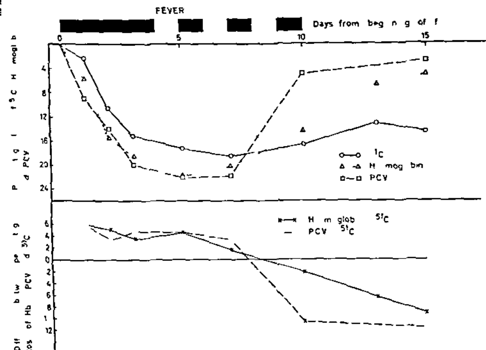


Fig 3 Upper curve Loss of Cr activity and reduction in haemoglobin and PCV in relation to pre febrile values during and after a ten day period of pyrogen-induced fe er (mean values from 3 rabbits)

Lower curve The difference in the relati e changes in haemoglobin or PCV and Cr activity during and after the fever

against the bacterial pyrogen afebrile days were interposed in the latter part of the fever period The result is presented in Fig 3 which shows the mean valu-s of fever induced ¹Cr loss in per cent at different times during and after the fever in three animals The rate of loss is seen to be greatest initially and after approximately 4 days of fever there occurs only a minor additional loss The total loss was in the same range as in the short lasting exp-riments

4 *Difference in the relative loss of ⁵¹Cr and haemoglobin or packed red cell volume*

Not only the percental loss of ¹Cr but also the percental fall in haemoglobin and packed cell volume (PCV) on the first day after the fever ceased for the three types of fever is seen in Table III The difference between haemoglobin and ⁵¹Cr loss and between PCV and ⁵¹Cr loss varied somewhat but on the average it was signif icantly above 0 There was no difference between the alteration in haemoglobin and PCV The find ings are even more distinct when the difference between the loss of haemoglobin and ¹Cr is followed during fever (cf Fig 3) The percental loss of haemoglobin exceeds the corresponding ¹Cr loss mainly at the start of the fever period but to a decreasing extent thereafter Calculated on the basis of 12 connected sets of values for the first 5 days the difference is significant ($p <$ 0 001) When regeneration of erythrocytes sets in as illustrated by the reticulocytosis in Fig 1 the values of haemoglobin and PCV return to normal and now the d viation of ¹Cr activity is greatest

5 *Relation between temperature response and erythrocyte loss*

Table IV demonstrates the temperature response expressed as Fever Index on each of the 5 days of fever as well as the sum of heat action obtained by the three methods of inducing elevated body temperature It is apparent that the later days of fever which contributed least to the loss of radioactivity and erythrocyte volume gave a ri e in the temperature of the same order as the first days

Fig 4 (right half) presents the relationship

Table IV *Temperature response in experimental fever in rabbits with bacterial pyrogen heated milk and external heating*
The response expressed as Fever Index (Δ degree C × hours)

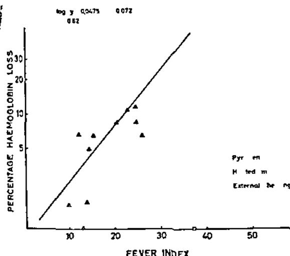
Method of fever	Dose/day (ml)	Animal no	Day 1	Day 2	Day 3	Day 4	Day 5	Total
Pyrogen	0 25	1B	8 4	12 7	13 2	11 7	3 7	49 6
	0 50	2B	4 5	8 5	10 0	12.5	4 0	39 5
	0 50	3B	16 5	10 0	14 1	15 3	13 2	69 1
	0 75	5B	12 9	12 4	17 2	17 6	12 8	72 9
	0 75	7B	7 0	8 3	11 7	12 6	10 5	50 1
	1 00	8B	6 3	6 3	7 5	6 6	5 3	33 3
	1 00	9B	7 7	7 0	12 1	11 6	8 1	46 5
	1 00	10B	6 1	5 9	9 9	9 0	6 9	37 8
	1 00	88A	12 0	14 1	15 5	15 0	13 2	69 8
	1 00	89A	3 6	6 3	10 2	12.6	11 9	44 6
	1 00	90A	6 1	8 8	13 0	12 7	14 2	54 8
	1 75	91A	5 8	4 9	4 6	6 4	4 5	26
	1 75	92A	7 5	15 9	20 3	19 6	19 6	82.9
	1 75	93A	8 9	13 3	[illegible]0 0	25 6	23 0	90 8
	2 50	94A	7 3	8 5	10 6	9 6	5 7	41 7
	2 50	96A	6 3	7 9	16 6	16 0	13 6	60 4
Mean			7 93	9 41	1[illegible] 89	13 33	10 64	54 24
± S D			± 1 30	± 3 30	± 4 25	± 4 86	± 5 62	± 18 45
Heated milk	5 00	14B	7 9	10 0	12 9	14 1	18 4	63 3
	7 50	23B	10 7	13 1	10 6	25 2	8 5	68 1
	7 50	24B	10 0	9 6	13 7	21 7	20 0	75 0
	10 00	25B	8 3	7 2	15 2	12 5	8 9	52 1
	10 00	26B	11 2	11 9	12 5	22 8	18 9	77 3
Mean			9 62	10 35	12 98	19 27	14 93	67 16
D			± 1 45	± [illegible] 26	± 1 68	± 5 57	± 5 77	± 9 68
ernal heating		12B	16 5	18 7	19 1	16 8	25 8	96 9
		13B	18 7	21 4	17 5	16 3	11 1	85 0
		30B	19 9	25 1	24 6	21 3	17 4	108 3
		42B	15 2	19 9	19 8	17 5	15 3	87 7
Mean			17 58	21 23	20 25	17 97	17 40	94 48
± S D			± [illegible] 11	± 2 77	± 3 08	± 2 27	± 2.15	± 10 5[illegible]

between temperature response and the percental loss of haemoglobin (logarithmic scale) after 5 days of fever induced by the three methods There seems to be a certain correlation corresponding to the small heat actions while above a Fever Index of about 60 a nearly constant loss of haemoglobin was seen Analysis of the relationship between temperature response and loss of haemoglobin after 2 days of fever (Fig 4 left half) shows that the results fall into two groups In the experiments using bacterial pyrogen and heated milk there is a positive correlation between temperature response and the logarithm of the percental loss of haemoglobin although not particularly good When temperature was raised by external heating on the other hand a loss of haemoglobin was not demonstrated until at somewhat higher Fever Indices

It must be emphasized that no correlation was found between the quantity of pyrogen administered and the loss of haemoglobin

II Studies by the DF³²P Method

The effect of pyrogen induced fever was studied on 6 rabbits after labelling of erythrocytes with DF³² P The period of fever was started 20 days after the labelling and was continued for 6 days Fig 5 shows the percental reduction of DF³² P and PCV in relation to fever the loss of activity being determined on the same lines as in the ⁵¹Cr experiments The change in activity was at a maximum just after the cessation of fever and was, on the average greater than in the ⁵¹Cr studies (26 78 % as compared with 14 14 % $p < 0\ 025$) The difference between observed and expected normal activity decreased in the same way as stated



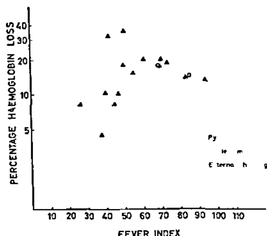


Fig 4 Relation between fever response (Fever Index = Δ degree C × hours) and relative reduction of haemoglobin (logarithmic scale) in fever induced by bacterial pyrogen heated milk or external heating

Left Fever for two days there is a correlation between Fever Index and the loss of haemoglobin in experiments with pyrogen and milk.

Right After five days of fever the haemoglobin loss appears to reach a maximum.

previously after the fever period In contrast to the ⁵¹Cr studies the reduction in PCV although in the same range as in the ⁵¹Cr studies ($p > 0.40$) was significantly less marked than the loss of activity during the entire period of fever ($p < 0.01$)

DISCUSSION

Alterations in the values of haemoglobin and PCV in connection with short lasting erythrocyte damage must be considered a priori to be less suited for quantitative determination of the magnitude of destruction of red cells since as a matter of course these parameters must be the resultant of destruction and of possible compensatory regeneration Instead it seemed likely that changes in erythrocyte survival curves after labelling with ⁵¹Cr and DF³²P might form the basis for such calculation as it is assumed that activity released by erythrocyte breakdown cannot be re utilized

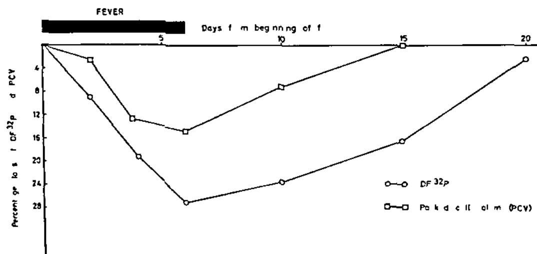


Fig 5 Loss of DF³²P activity and the reduction in PCV in relation to pre febrile values during and after a six-day period of pyrogen induced fever (mean of 6 experiments) The fever was initiated twenty days after the labelling of the erythrocytes

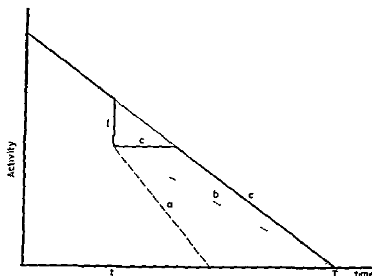


Fig 6 Theoretical cour es of erythrocyte survival after a *transitory destruction* of red cells (shown by line *l* at time *t*) depending upon the type of cell injury

Course a Injury to all red cells, some being destroyed at once and the remainder dying before normal life span (*T*)

Course b Acute random destruction with no injury to the surviving cells the slope is less steep and the last labelled ce l leaves the blood at time *T*

Course c Preferentially destruction of the older cells with no injury to the surviving cells In the first period after the destruc tion no labelled cell will leave the blood

The difficulty consisted in finding a basis for assessment which would render it possible to con vert the demonstrated alterations in curves to an expression of the increased haemolysis

Under normal conditions the erythrocyte break down in rabbits is due to a combination of senes cence and random destruction Since due to the latter type of destruction there is a skew age *distribution function the equation representing the* loss of tracer labelling the entire erythrocyte popu lation must be rather complicated (3 4)

$$\frac{N_t}{N_0}=\frac{e^{-bt}-e^{-bT}}{1-e^{-bT}} \qquad (1)$$

(N_t = activity at time t N_0 = activity at time 0 b = exponential coefficient for random destruction T = maximum erythrocyte life span) Labelling with Na $^{51}CrO_4$ introduces one more unknown owing to elution of Cr from intact erythrocytes

$$\frac{N_t}{N_0}=\frac{(1-e^{-b(T-t)})e^{-(b+k)t}}{1-e^{-bT}} \qquad (2)$$

(k = exponential coefficient for elution of Cr)

However these theoretical equations are appli cable only to the pre febrile spontaneous segment of the present erythrocyte survival curves Accord ingly a simplified model was introduced in ana lysing the experimental results utilizing the fact that small segments of the survival curves may be interpreted approximately as a straight line in a semilogarithmic system The fever induced change in the curve is demonstrated partly by the steeper slope of the segment during the fever partly by the difference of the observations from a theoreti cal curve plotted by extrapolation of the pre febrile segment which represents the pattern of spon taneous loss of activity to the subsequent time units A comparison between the different aberra tion of the observations in a control group and in the fever exposed groups from this hypothetical curve afforded an expression of the percental loss *in activity in relation to that expected during and* after the fever

By both tracer methods and by all three methods for experimentally inducing elevation of body temperature the study showed an unmistakable fall in activity in the circulation during fever This loss coincided with a reduction in haemoglobin con centration and PCV The maximum loss o curred soon after the environmental change had been induced and at continued el vation of temperature the loss of activity per time unit was in the same range as under normal conditions When the fever ceased the difference between the expected normal and the actually observed course of the erythrocyte survival curve decreased gradually The explana tion for this is presumably to be found by inspect ing the theoretically possible courses of the survival curve after a transitory action which entails a cer tain loss of activity In Fig 6 the normal survival curve is simplified as a straight line At time *t* the animal is exposed to an abrupt loss of erythrocytes corresponding to segment l If the action upon the erythrocytes is of a nature which affects all cells of the population irrespective of age in such a way that some cells die immediately and the re

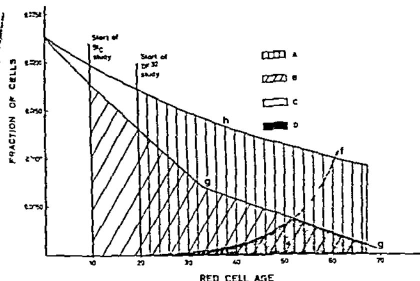


Fig 7 Influence of a short period of fe er on the erythrocyte popu.ation in rabbits il ustrated on the basis of the distribution of red cells as a function of cell age (calculated f om equation (3) $b = 0.0135$ and $T = 67$) Abscissa different ages of red cells Ordinate fraction of cells at respectı e a es. Total area total erythrocyte population = total haemog.obin mass.

Area A Part of popu.ation labelled by DF³²P at the beginning of feve period (twenty days after labellin) *f* Tentat ve line showing age-dependent difference in sensitivity to fever This line cuts out area *C* which represents about 26% of area *A* and about 15% of total area (the relative reduct ons in DF³²P and haemoglobin respectively found in the study)

Area B Part of population labelled by ⁵¹Cr at the beginning of fever period (ten days after labelling) This area is limited by a tentative line (*g*) representing difference in labelling intensity as a function of cell age (cf. text)

Area D The ⁵¹Cr l belled fraction of cells lost during fe er Depending upon the course of line *g* area *D* expressed in per cent of area *B* may be greater or smaller than the haemoglobin lost, represented by area *C* in per cent of total area under line *h*

mainder die at varying time but earlier than expected, the last labelled erythrocyte will leave the blood before the time of normal life span (*T*) in accordance with course *a* If the damage involves random destruction, i e does not alter the chance of the remaining cells of obtaining the usual life span the curve after the cessation of the damaging action will present a flatter slope than normal and intersect the time axis at *T* (course *b*) If, however the damage only involves old erythrocytes and the unaffected cells continue to have a normal life span, there will be no labelled cells destined to break down during the period immediately after the fever thus the curve will be horizontal until it reaches the curve representing the normal course which it will accompany as far as *T* (course *c*) The course of the survival curves found in the present study after the fever period appear to fit in best with possibility c the observed and expected normal course apparently coinciding before the normal life span of rabbit erythrocytes (about 60 days) This is in keeping with previous experiments (6) using two-generation labelling with two iron isotopes which clearly showed that the erythrocytes are not destroyed by fever until they are about 20 days old or more that the destruction increases with advancing cell age and that the changes could not be explained by haemodilution The quantitative difference between the experiments using ⁵¹Cr and DF³²P may then be explained by this rise in sensitivity with increasing cell age The latter isotope at the time of fever is present in relatively older cells than the former and the reduction in activity will be greater in spite of practically identical change in PCV

An attempt to explain the demonstrated differences in the relative reductions in radioactivity and haemoglobin or PCV is given in Fig 7 This illustrates the total red cell population distributed in fractions with cells of different age The equation for this function is derived from equation (1) by differentiation

$$a(t)=\frac{b\ e^{-bt}}{(1-e^{-bT})} \quad (3)$$

($a(t)$ = fraction of cell mass with age = t)

In this case the constants $b = 0\,0135$ and $T = 67$ were used according to Brown and Eadie (1) As expected there is a decrease in number of cells with increasing age due to random destruction. The total area in the figure is an expression of the entire red cell population and equals 1 In Fig 7 moreover a tentative line (*f*) is plotted for sensitivity to fever as a function of erythrocyte age starting at age 20 and showing the demonstrated increase in sensitivity with advancing age (6) In the experiments using DF³ P the isotope is present in cells which are at least 20 days of age at the initiation of fever (area A) The experimental fever period has given a mean disappearance of 26 per cent of DF³ P present and if a corresponding area (*C*) is cut out, using the line representing sensitivity due to cell age this will remove approximately 15 % of the entire area under the curve which is measured as haemoglobin or PCV These numbers are in accordance with the relative reductions in respectively DF³ P and PCV found in the present study The same reflections might be [illegible]ed to the experiments using ⁵¹Cr At the onset [illegible] fever the labelled cells are at least 10 days old and if the ⁵¹Cr did represent the total cell mass with ages above this limit one would a priori expect a difference between the relative changes in radioactivity and haemoglobin or PCV in the same direction as in the DF³ P experiments although less marked The experimental findings however are exactly opposite as the mean value for relative loss of haemoglobin or PCV exceeded the ⁵¹Cr loss especially during the initial part of the fever period. Studies have been made however which would indicate that the ⁵¹Cr method does not label the erythrocyte population uniformly Thus differential centrifugation with coarse separation of the erythrocytes into various age groups has shown that the youngest cells contain twice the ⁵¹Cr of the average population and 3–4 times that of the content in the oldest cells (2) This difference of labelling intensity with various cell age has tentatively been illustrated by line *g* in Fig 7 assuming that all the youngest cells are labelled, and depending upon the detailed shape of this function the fever induced loss of the ⁵¹Cr labelled part of the erythrocyte population (area *D*) expressed in per cent of total ⁵¹Cr area (area *B*) may be sometimes greater and sometimes smaller than the removed haemoglobin (area *C*) amounts in per cent of the total haemoglobin mass, as was seen in the present study (Table III)

From the difference in the results obtained with ⁵¹Cr and DF³ P it is seen that the loss of activity in simple consequence of the age-dependent variation in sensitivity depends upon the placement of the fever period in relation to the time of labelling As far as the ⁵¹Cr method is concerned the loss depends as just mentioned also upon the presumed individual variation in the labelling intensity with different cell age The measured changes in activity therefore served mainly to elucidate the erythrokinetics during fever while the best obtainable quantitative expression of the loss in the erythrocyte mass in the present study is the decrease in haemoglobin and PCV The changes of these parameters will be underestimated if appreciable regeneration occurs during the fever

Judged by the effect after a five-day period of fever there seems to be a maximum limit to the loss of haemoglobin (Fig 4) This accords with the chronological course of the loss during the fever it was shown that the greatest loss occurs at an early stage of the fever period and that a prolongation of the elevated temperature does not contribute much to the reduction Considering the situation after a short time of action (2 days) there is a fairly good correlation between Fever Index and haemolysis in pyrogen and milk induced fever the loss of haemoglobin appears to increase exponentially with increasing heat action, and there does not seem to be any threshold of susceptibility The erythrocyte sensitivity is apparently less when elevated body temperature is induced by external heating This may be due to a difference in size between core and rectal temperature during external heating and during the "internal" methods of fever

CONCLUSION

On the basis of the present analysis the following conclusions may be drawn Elevated body temperature during experimental fever is followed by a decrease in the mean survival time of the erythrocytes and according to this the total mass of erythrocytes will soon be to some extent reduced. In the rabbit this loss makes up about 15 % of the

red cells The further course depends upon whether the bone marrow under the new environmental conditions is able to compensate for this loss If the bone marrow can produce erythrocytes only at the normal rate the animal will remain anaemic to an extent corresponding to the primary loss of red cells Otherwise erythropoiesis has to be increased in step with the reduction of mean survival time In addition the bone marrow must produce a quantity of red cells corresponding to the primary loss

When using the previously selected constants for the breakdown equation a loss of 15% of the red cell mass from the older part of the population corresponds to a reduction in mean cell survival time of from 44 to 37 5 days Correspondingly the daily percental influx from the bone marrow has to increase from 2 26 to 2 66 i e an increase of 18% With the maximum erythrocyte loss of about 35% which was demonstrated in the present material the mean survival time will be 28 7 days and the daily production required to maintain a normal haemoglobin level will be 3 48% of the erythrocyte mass an increase of 54% The increased demands on the bone marrow must be said to be modest compared with its normal capacity but may be important in diseases involving relative inhibition of the bone marrow

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DOUBLE BLIND TRIAL OF LONG TERM ANTICOAGULANT TREATMENT AFTER MYOCARDIAL INFARCTION

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Abstract A prospective double blind trial of long-term anticoagulant medication following myocardial infarction was carried out in Utrecht from May 1 1964 to January 15 1966 With the collaboration of all Utrecht cardiologists, all their patients with acute myocardial infarction were included in this trial after a 4-month interval to be distributed strictly at random over a phenprocoumon and a placebo group Supplemental therapy and follow up were identical in both groups Of the total of 138 patients included in the trial, 68 received phenprocoumon and 70 were given a placebo One patient died in the phenprocoumon group and eight patients died in the placebo group This difference is significant ($P < 0.01$) The difference in the number of reinfarctions was not significant (5 cases and 7 cases respectively)

After January 15 1966 when the difference between the two groups had become significant all patients were further treated with phenprocoumon. Nevertheless, the difference in mortality continued through 1966 with five deaths in the original placebo group and only one death in the original phenprocoumon group

The mean thrombotest level was 10 7% patients remained within the therapeutic limits (5-15% thrombotest) during an average of 91% of the entire period of investigation. The mean daily phenprocoumon dose was 3.1 mg. No serious haemorrhagic complications occurred

The results of our study are consistent with those of several other recently published investigations. The intensity of the hypocoagulability attained is probably of decisive importance in this respect. However correct calculation of the mean level of hypocoagulability is difficult.

The fact that in the original placebo group the results remained less favourable even after the switch to phenprocoumon emphasizes the necessity of long term treatment with adequate doses of anticoagulants following myocardial infarction

The value of long term oral anticoagulant medication in coronary insufficiency has been a controversial and emotion laden subject since the early years of this decade In 1965 Merskey and Drapkin (18) concluded in an excellent review that the data then available warranted no definite statement concerning the validity of this therapy

In view of the risks and inconveniences entailed by long term or even life long anticoagulant medication we considered it very important to establish the validity of this therapy in more unequivocal terms To achieve this goal we instituted a strict double blind trial of long term oral anticoagulant medication after myocardial infarction with the collaboration of all cardiologists practising in Utrecht and of the Utrecht Thrombosis Service

MATERIAL AND METHODS

A Choice of patients

The investigation started on May 1 1964 and was concluded on January 15 1966 at which time the material totalled 138 patients (124 men and 14 women) of whom 68 received anticoagulants while 70 were given a placebo The total months of trial exposure was respectively 1317 and 1474 months.

Our study covered *all* patients in whom, since May 1 1964 a specialized cardiologist had definitely diagnosed either myocardial infarction or its recurrence on the basis of clinical, biochemical and ECG evidence Once the diagnosis was established all were treated with acenocoumarol (Sintrom® Geigy) until their entry into the trial.

The patients were included in the trial procedure as soon as they were fully ambulant, i.e after an average of four months Data recorded for each patient included height and weight, age sex, localization of the infarct and the occurrence of a drop in blood pressure and or arrhythmia the degree of peripheral vascular sclerosis, blood pressure blood sugar and family history the record also described the patient s condition at entry fitness, cardiac decompensation, angina pectoris, ECG and therapy instituted

All patients were given numbers in the order of their appearance Only this number determined whether a given patient was to receive the anticoagulant or the placebo

Table I *Principal data on the composition of the phenprocoumon and the placebo group*

		Number of patients	
		Phenprocoumon	Placebo
Age distribution y			
Men	< 50	14	13
	50–59	28	29
	60–69	18	21
	70	1	—
Women	50–59	3	3
	60–69	4	4
Total		68	70
Average age		55 5	55 8
Total months of exposure		1317	1474
First infarction		63	62
Reinfarction		5	8
Severity of infarction			
Mild		34	30
Moderate		26	29
Severe		8	11
Localisation of infarct			
Anterior wall + spread (if any)		34	27
Other sites		34	43
Angina pectoris at entry			
Mild		17	17
Moderate		20	26
Severe		6	3
Cardiac decompensation		2	0
Peripheral vascular sclerosis			
Mild		17	17
Moderate		20	26
Severe		6	3
ability			
Moderate		37	37
Severe		3	2
Diabetes mellitus		3	3
Overweight		16	16
Suggestive family history		9	9

For this purpose a random list had been made prior to the trial this list stipulated for each number whether anticoagulant or placebo was to be given In this way we ensured an entirely random distribution of patients. The principal data on the comparability of the two groups of patients are presented in Table I More detailed information can be supplied to the interested The interval between the infarction and the entry into the trial is given in Table II

B *Anticoagulant medication*

The anticoagulant used in the trial was phenprocoumon (Marcoumar® Hoffman La Roche Basle) the same manufacturer supplied the placebo-phenprocoumon tablets, which were identical to the phenprocoumon tablets in shape colour and taste All tablets were dispensed by the nurses of the Thrombosis Service Neither the patient himself nor the family doctor attending cardiologist or nurse were informed of the nature of the tablets the patient received. Only the project leader was aware of

Table II *Comparability of the two groups as to interval between infarction and entry into the trial*

	Interval in months							
	<3	–4	–5	–6	–7	–8	–9	Mean
Phenprocoumon	8	34	16	5	2	2	1	4 0
Placebo	2	27	20	15	5	1	0	4 5

this The patients were given to understand that they participated in a study concerning tablets of a new type

It was ensured that therapy group and placebo group were identical as to fluctuations in dosage and frequency of blood sampling for laboratory studies Contrary to convention coagulation studies were not performed in the Thrombosis Service Laboratory lest the nature of the tablets become known and nurses be informed of the group to which the patient belonged Determinations were instead made in the coagulation laboratory of the Utrecht University Medical Clinic One of us (O J A T M) then informed the Thrombosis Service of the dosage to be employed and the date of reporting for the next follow up The Thrombosis Service provided patients with the usual calendar showing the number of tablets to be taken each day and the new date of examination Records were kept of the number of tablets dispensed The number of tablets left to the patient at the end of each period indicated whether instructions as to dosage had been followed

The phenprocoumon dosage was controlled with the aid of the thrombotest method (19) The goal was a dosage which confined hypocoagulability to between 5% and 15% thrombotest For calculation of the mean dosage level we calculated the level of hypocoagulability for each patient for each day between all consecutive follow-ups, as a mean between the preceding and the subsequent thrombotest determination. On the basis of all these daily results the mean level of hypocoagulability was calculated over the entire period of investigation for the entire

Table III *Incidence of unbalance of phenprocoumon dosage*

No of pats	No of times unbalanced
11	0
17	1
23	2
6	3
7	4
2	5
0	6
1	7
1	8
68	134

Mean per patient 1 97 times

Table IV *Locali ation of bleeding complications and thrombotest value during tl e bleeding episode*

Localization	Thrombotest ( )
Subconjunctival	5[a]
Subconjunctival	9[a]
Subconjunctival	6
Subconjunctival	5
Nasopharyngeal	14
Urogenital	3
Urogenital	7
In est nal	10

[a] Same pat ent

material The mean level thus calculated was found to be 10 7% thrombotest. Patients were regarded as unbalanced in terms of anticoagulant therapy when the thrombotest value was not between 5% and 15% Throughout the period of investigation, hypocoagulability was unbalanced an average of 1 97 times per patient (Table III) Per patient, the thrombotest value was between 5% and 15% during an average of 91% of the total period of investigation. The mean Marcoumar dosage per patient per day was 3 21 mg

A total of e ght haemorrhages occurred in seven patients (Table IV) This averages out at one haemorrhage per patient per thirteen years of treatment. These were all patients in the phenprocoumon group An overdosage played a role in only one case In no case was it necessary to interrupt phenprocoumon medication for a considerable length of time In view of excessive diminution of the *thrombotest value* 15 patients were given vitamin K (Konakion® Hoffman La Roche) in one patient this was required on two separate occasions None of these patients showed signs of a haemorrhagic diathesis In three patients, administration of tablets was temporarily discontinued in view of indispensable dental therapy Three patients were hospitalized for other reasons in the course of the trial None of the abovementioned facts concern deceased patients

C *Cont aind cations to participation*

We excluded from the trial all individuals for whom one or several of the following contraindications were valid.

1 Age over 70
2 Diastolic pressure exceeding 1 0 mm Hg.
3 Severe hepatic and/or renal insufficiency
4 Diseased associated with increased risk of haemorrhage (active peptic ul er haemorrhagic diathesis, etc)
5 Concomitant malignant disease (carcinoma, etc)
6 Valvular abnormalities and/or atrial f brillation
7 *Asocial behaviour and/or insuff cient intelligence*

D *Cont ol of patients*

The attending cardiologist examined the patient at least every three months during the first year and subsequently at least every six months Whenever a recurrence of myocardial infarction was suspected an ECG was recorded and one or several blood samples were obtained for determination of SGOT SGPT and CPK activities After each examination the cardiologist submitted to the project leader a follow up form stating the patient s subjective and objective condition the number of glyceryl trinitrate (Nitrobate) tablets used, ECG changes, results of biochemical determinations, if any and a general evaluation of improvement or deterioration in the patient s cond tion.

In the case of a recurrence of myocard al infarction, the decision to start or withhold anticoagulant therapy was taken before the nature of the tablets used by the pat ent was disclosed If anticoagulant therapy was indicated, this

Table V *Review of deceased patients*

Placebo (P) Phenprocoumon (M)	Age (y)	Sex	Infarction ranking	Clinical impression of infarction	Cholesterol (mg/100 ml)	Infarct localization	Angina pectoris at entry	Peripheral vascular sclerosis	Fitness at entry	Diabetes mellitus	Obesity	Interval between entry and death (days)
P	54	♂	1	M ld	214	Ant rior wall	Absence	Severe	Und sturbed	No	Yes	352
P	60	♂	1	Mild	272	Lateral wall	Moderate	Se ere	Moderate	No	No	156
P	59	♂	1	Mild	?	Inferior wall	Mild	Moderate	Undisturbed	No	No	182
P	58	♂	2	M ld	280	Anterior & infer or wall	Moderate	Moderate	Moderate	No	No	160
P	67	♀	1	Moderate	169	Anterior & lateral wall	Mild	Moderate	Moderate	No	No	10[illegible]
P	50	♂	1	Moderate	203	Lateral & posterior wall	M ld	Absent	Moderate	No	No	335
P	45	♀	1	Severe	186	Infer or wall	M ld	Absent	Moderate	No	No	278
P	61	♀	1	M ld	440	Lateral & posterior wall	Absent	M ld	Undisturbed	Yes	Yes	173
M	58	♂	1	Mild	280	Anterior wall	Absent	Moderate	Undisturbed	Yes	No	98

Table VI *Comparison between deceased and living patients*

	Total no of pats	Deceased	Living
Male	124	8	116
Female	14	1	13
Mean age y	55 5	57 4	55 4
Hypertension			
Absent	116	8	108
Present	22	1	21
Overweight			
Absent	98	7	91
Present	40	2	38
Diabetes mellitus			
Absent	132	7	125
Present	6	2	4
Disability			
Absent	59	4	55
Moderate	74	5	69
Severe	5	0	5
Infarct localization			
Anterior wall with spread (if any)	61	4	57
Other sites	77	5	72

was given for at least three months whereupon the patient was returned to the therapy he had received prior to the recurrence

Evaluation of results

criteria to be used in evaluating results were for lated before the trial was started namely

mortality

2 number and severity of recurren es of infarction

3 subjective and objective changes in the patient s con dition as described on follow up forms (see above) by attending cardiologists ignorant of the nature of the tablets used by their patients

RESULTS

On January 15 1966 a total of nine patients had died eight of the deceased were in the placebo group and one was in the phenprocoumon group

The principal data on the deceased patients are presented in Table V Of the eight placebo pa tients six died a peracute death at home while the remaining two died a few hours after hospitaliza tion for recurrence of myo ardial infarction The patient from the phenprocoumon group died as a result of colisepsis in prostatitis no fresh myocar dial changes were found at the postmortem

The difference between the two groups is highly significant ($P < 0.01$) Comparing the deceased with the non-deceased in our small material we find no demonstrable influence of sex age hyper tension overweight and infarct localization of the six patients suffering from diabetes mellitus two died (Table VI)

Table VII *Comparison of mortality with predicted mortality in The Netherlands population during the period May 1 1964 to Jan 15 1966*

	Total no of pats	Deceased per Jan 15 1966	Pre dicted value	Tail proba bility in test ing for over mortality
Phen procoumon	68	1	1 7	0 45
Placebo	70	8	1 7	0 001

Table VII compares the death risk in the group studied with that of a Netherlands population of comparable age distribution The comparison shows that the death rate in the placebo group is signifi cantly higher than could be expected in view of the mortality in the Netherlands population This is not the case in the phenprocoumon group

The survival of the deceased patients after entry into the trial averaged 204 days (Table V) The shortest survival in the placebo group was 102 days and consequently there are no indications suggesting that abrupt discontinuation of Sintrom medication at entry into the trial has led to reac tive hypercoagulability with increased thrombotic tendency

There was no significant difference in number of recurrences of myocardial infarction between the placebo group (7 patients) and the phenpro coumon group (5 patients)

Of the patients in the phenprocoumon group 52 % felt improved and 13% felt deteriorated corresponding values in the placebo group were 42% and 14% In the cardiologist s judgement 30 of the phenprocoumon patient showed im provement and 12 showed deterioration cor responding figures in the placebo group were 21% and 15% (Tables VIII and IX)

Table VIII *Subjective changes in the patient s condi tion (% of all follow up forms)*

	Better	Unchanged	Worse
Phenprocoumon	52	35	13
Placebo	42	44	14

Table IX *Objective changes in the patient s condition* (o *of all follow up forms*)

	Better	Unchanged	Worse
Phenprocoumon	30	49	12
Placebo	21	64	15

Table X *Comparison of mortality with predicted mortality in The Netherlands population during the period Jan 15 1966 to Jan 1 1967*

	Total no of pats	Deceased per Jan 1 1967	Predicted value	Tail probability in testing for over mortality
Phenprocoumon	65	1	0 9	0 59
Placebo	62	5	0 9	0 02

When the difference between the phenprocoumon and placebo groups had become significant on January 15 1966 the trial was discontinued and all patients were adjusted to phenprocoumon Follow up system dosage and supplemental treatment remained unchanged Although the original placebo patients were now treated with phenprocoumon the mortality in this group remained significantly higher than in the original phenprocoumon group (Table X) The difference in mortality was not only demonstrable during the first few months after the change to phenprocoumon three of the five deaths of patients from the original placebo group occurred in November and December 1966

DISCUSSION

Since Wright s publi ation in 1948 (28) on long term anticoagulant medi ation following myocardial infarction this therapy has been the subject of numerous studies Several reviews have summarized the results of these investigations (12 13 17 18 23 27)

In their review Merskey and Drapkin (18) in 1965 formed the conclusion that none of the papers thus far published had reported on studies which met requirements of optimal strictness Such authors as Douglas (7) formulated the requirements to be m t by such investigations as follows Double blind technic random allocation of subjects into treated and control groups determination of suitability for anticoagulant therapy prior to allocation into treated and control groups identical therapeutic regimen in both groups except for the anticoagulant drug and objective criteria such as death rate for determining the value of the therapy

Many investigators in The Netherlands and abroad (8 9 11) still doubt the value of long term or life long oral anticoagulant medication Prior to our trial we too felt very doubtful concerning the validity of long term therapy The majority of the participating cardiologists were accustomed to confining anticoagulant therapy to the first three months after myocardial infarction Many patients in our ph nprocoumon group therefore received anticoagulant therapy for a longer period than would have be n the case had they not b en included in the trial

In view of the design and procedure of our trial (see Methods) we believe that we have met all the requirements which can be reasonably formulated for studies of this type Our study was double blind Our material can be considered representative for Utrecht and its environs because all Utrecht based cardiologists participated in the trial The study covered all their patients with myocardial infarc ion panel as well as private patients The cardiologists diagnosed myocardial infarction and/or its recurrence on the basis of clinical biochemical and ECG findings Allocation to therapy of control group was strictly at random simply on the basis of numbers which the patients were given in the order of their appearance For each number it had been decided in advance whether phenprocoumon or a placebo would be given

The contraindications to participation—e g severe hypertension haemorrhagic diathesis etc — cannot have influenced the distribution of patients over therapy and control groups for the patient was not allowed to enter the trial until he met pre formulated criteria

Suplemental therapy and follow up system were fully identical for the two groups Prior to the trial mortality had been accepted as the principal criterion in evaluating results

In patients admitted to the trial recurrence of myocardial infarction was diagnosed before the attending cardiologist knew whether the patient in

question was in the phenprocoumon or in the placebo group

Loeliger (14) demonstrated the great importance of adequate dosage in long term anticoagulant medication In comparing the results of various studies therefore great importance must be at tached to the mean level of hypocoagulability However calculating this level is a precarious undertaking A primary disadvantage is the pres ence of an asymmetrical distribution of thrombo test results above the desired level the range is from 15 % to 100 % thrombotest while below this level the range is only from 5 % to 1 % throm botest This leads to a rise of the mean thrombotest level and consequently suggests a less favour able level of hypocoagulability

If the mean dosage level is calculated not on the basis of thrombotest percentages but on the basis of clotting times determined and if the mean clotting time established is then expressed in thrombotest percentage then the asymmetrical distribution which likewise characterizes the clot ting times leads to a low thrombotest percentage suggesting a very favourable level of hypocoagula bility

A second disadvantages lies in the fact that pa whose values are above or below the desired of hypocoagulability are likely to be more examined in follow up than patients who are firmly balanced at the desired level Therefore the frequency of follow up on balanced patients and the frequency of follow up on un balanced patients are factors which in part deter mine the mean level of hypocoagulability if this is calculated as the mean of all thrombotest results obtained or of a random sample of results In order to avoid this second disadvantage we cal culated—or each patient for each day between all successive follow ups—the level of hypocoagula bility as the mean of the preceding and the sub sequent thrombotest result (not the clotting time!) On the basis of all these day averages we cal culated the mean level of hypocoagulability for the entire period of investigation and the entire material The mean level of hypocoagulability thus calculated was 10 7 % thrombotest

So far as we know this method of calculation has not been used by other investigators even so the problem of an asymmetrical distribution of thrombotest results persists and the level of hypocoagulability attained is therefore perhaps best compared on the basis of the mean dosage of anti coagulant used

The mean phenprocoumon dosage per patient per day in our trial was 3 21 mg Loeliger (14) found a mean daily dosage of 2 83 mg This mode of comparison too has its disadvantages e g the dosage is dependent on dietary habits climatological influences and the patients average age However the advantage that the mean dosage can be calculated in a simple and identical way by all concerned is not negligible

Phenprocoumon was the anticoagulant used In long term anticoagulant medication we prefer a long acting anticoagulant because our findings and results show like other investigations (3 4 6 22) that a much more stable balance can be achieved with a long acting anticoagulant The thrombotest value per patient was not between 5 % and 15 % thrombotest on only 1 97 occasions the patients were within the proper therapeutic limits for an average of 91 % of the entire period of investiga tion

Much sooner than we expected we were forced to discontinue the trial because the difference in mortality between therapy group and control group had become significant ($P < 0.01$) even in our relatively small series One death in the phen procoumon group compared with 8 deaths in the placebo group The deceased included patients who were younger as well as patients who were older than 55

Our small series warrants no definite con lusion as to the influence of sex age overweight fitness at entry and infarct localization on the prognosis however it is a conspicuous finding that two of the six patients with diabetes mellitus died

The mortality in the phenprocoumon group does not differ from that in the Netherlands population of comparable age distribution The difference in this respect between the placebo group and the Netherlands population is highly significant ($P < 0.001$)

The number of reinfarctions were relatively small in this respect the difference between the phenprocoumon and the placebo group was not significant (reinfarction occurred in 5 and 7 cases, respectively)

The two groups did not significantly differ either in subjective or objective changes in the patient s condition This was evaluated on the basis of quarterly reports submitted by the parti

cipating cardiologists who described subjective and objective changes in the patient's condition without knowing to which group the patient in question belonged. Of the phenprocoumon patients 52% felt improved while 13% felt deteriorated, the corresponding figures in the placebo group were 42% and 14% respectively. In the cardiologist's opinion improvement was noticeable in 30% of phenprocoumon patients, and deterioration in 12%, the corresponding figures in the placebo group being 21% and 15% respectively.

Thus while neither the number of reinfarctions nor the subjective and objective changes in the patient's condition differed significantly in the two groups, yet in these respects too the phenprocoumon group showed the best results.

The highly significant difference in mortality found in our trial between therapy group and control group within so short a time is surprising for the majority of studies using a comparable control group disclosed no difference in mortality. The British Medical Research Council (20, 21), Aspenstrom (1), Harvald (10), MacMillan (16) and Seaman (24). Bjerkelund (2) found a diminished mortality only in patients under 60, and Clausen (6) observed it only in patients under 55—and only during the first year following the infarction. The absence of a difference in mortality in a number of these studies is probably explained in part by less adequate hypocoagulability (14).

Our findings are consistent with those reported by Lovell (15) and Schnapper (26) who likewise observed diminished mortality in their therapy group. Loeliger (14) also found a difference in mortality in favour of his therapy group although in his myocardial infarction trial this was not significant. But if his data are combined with those of a simultaneously made double blind study of peripheral arteriosclerosis, then the difference found by him is significant also. In this context it must be pointed out that patients were not included in his trial until at least a year after they had suffered an infarction.

After January 15, 1966 all our placebo patients were switched to phenprocoumon about halfway through March 1966 all these patients had attained an adequate level of hypocoagulability. Follow up and supplemental therapy were continued in the same way. On January 1, 1967 the number of deaths in the original phenprocoumon group had risen to two, and that in the original placebo group had increased to 13. This difference too is significant. This finding is the more surprising because the higher mortality in the original placebo group had led to selection to the disadvantage of the phenprocoumon group. The fact that, after return to anticoagulant medication, the results in patients deprived of this therapy for a considerable period continue to be less favourable stresses the necessity of protracted and adequate anticoagulant therapy following myocardial infarction.

Our findings supply no answer to the question as to how long this medication should be continued but—also in view of recent data from the literature—we believe that very protracted or even life long medication is indicated.

In the placebo group the Sintrom which the patients had received in the acute phase of myocardial infarction was replaced by placebo tablets within a day. This is a very abrupt discontinuation of anticoagulant medication. We have found no indications suggesting that this abrupt discontinuation gave rise to reactive hypercoagulability with an increased thrombotic tendency (25).

Addendum. Since the preparation of this paper two years have elapsed. Follow up phenprocoumon therapy and supplemental treatment were continued in the same way. From January 1967 to January 1969 three patients died in the original phenprocoumon group, in the original placebo group seven patients, making up a total mortality since the end of the trial of four and twelve patients respectively.

These results are very remarkable and intriguing and, as far as we know, have never been reported before.

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EVALUATION OF FINE NEEDLE ASPIRATION BIOPSY SMEARS IN THE DIAGNOSIS OF LIVER IRON OVERLOAD

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Abstract Estimate of stainable iron in fine needle aspira tion biopsy smears has been evaluated in relation to histological estimate of stainable iron in needle b opsy sections and in relation to liver iron in needle biopsy specimens analysed chemically Histochemical iron was arbitrarily graded 0-4 The histochemical grading in sections agreed well with that in smears, but there was a high degree of overlapping between the chemical iron values in the differ ent gradings However it can be concluded that the smear method is valuable as a simple screen n test in the diagnosis of l ver iron overload grade 4 in most cases indicates definitely increased liver iron concentration

In the diagnosis of haemochromatosis indirect evi dence of liver iron overload can be ascertained by several procedures such as uptake of radioactive iron in ferrokinetic studies or quantification of iron stores by chelating agents For exact diagnosis however a study of liver biopsy specimen is necessary The most reliable method is to analyse liver iron chemically which can be done on per cutaneous needle biopsy specimens (4) Conven tional needle biopsy technique (Menghini Vim Sil verman) however is not without risks and is therefore unsuitable as a screening test of liver iron overload

By the use of fine needle aspiration biopsy tech nique as described by Soderstrom (5) liver punc ture complications are virtually absent, and this type of biopsy can be used on wide indications without undue risks Stainable iron in such fine needle biopsy smears has been demonstrated (1 5) but no systematic evaluation of this method as a tool in the diagnosis of liver iron overload has been published In the present study the estimate of stainable iron in fine needle aspiration biopsy *smears* was evaluated in relation to the estimate of stainable iron in histological needle biopsy *sections* and in relation to liver iron in needle biopsy speci mens analysed chemically

MATERIAL

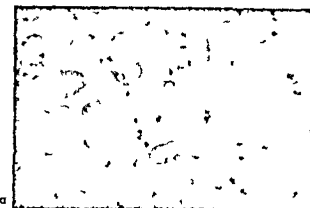
The study included 59 patients (53 men and 6 women) The biopsy indication was in most cases chronic alcoholism (33) or porphyria cutanea tarda (13) The rest of the patients were biopsied for suspected liver disease H stologically steatos s was found in most some had fibrosis of varying degree and some cirrhosis. In a few inflammatory changes dominated the histological picture

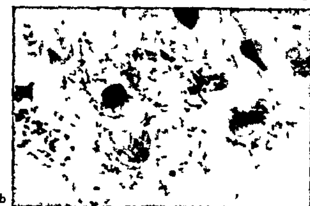
METHODS AND PROCEDURE

After local anaesthesia the puncture was made in the mid axillary line in a suitable intercostal space (usually the 9th) durin apnoe The fine needle aspiration biopsies were performed with the instrument designed by Franzén et al ( ) Needles with an outer diameter of 0 7 mm were used The aspirate was immediately distributed in thin short smears on several slides and allowed to air-dry The biopsy technique has been described in detail by Soderstrom (5) Directly after the fine needle biopsy aspiration biopsy with thicker needle (internal diameter 1 6 mm) was per formed. A small part of this biopsy specimen was fixed in 10% neutral formaldehyde for 24 hours, embedded in paraffin and cut into 3 μ sections for histological ex amination. Most of the biopsy specimen was used for chemical analysis of non haemin iron

The methods for staining haemosiderin in sections and for analys n nonhaemin iron and alkali-soluble protein were the same as described earlier (4) The stainin of ha mosiderin in smears was done by the method described by Hansen and Weinfeld (3)

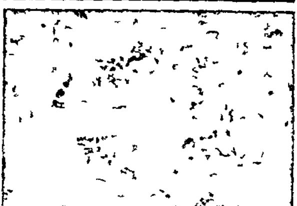
To assess the amount of histochemical iron in fine needle aspiration biopsy specimens, the smears were ex amined at 900× magnification under immersion (Fig. 1) Iron may be situated in parenchymal cells and Kupffer cells The latter in contrast to the former h er ar











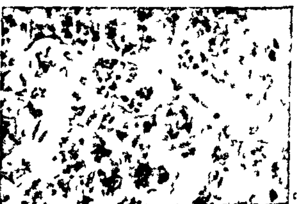


Fig 1 Fine needle aspiration biopsy smears showing iron granules in liver cells ×800 (*a*) Grade 2 (*b*) Grade 3 ( ) Grade 4

Fig 2 Corresponding findings in sections stained for iron ×800 (*a*) Grade (*b*) Grade 3 (*c*) Grade 4

very easily and by the smear technique phagocytized iron pigment is often spread extracellularly and is difficult to distinguish from artefacts Therefore in this study only parenchymal cell iron was estimated For fine needle aspiration smears the following grading was used

Grade 0 No stainable iron or only isolated fine granules in all smears

Grade 1 Fine granular haemosiderin in a minority of the cell groups.

Grade 2 Fine granular haemosiderin in a majority of the cell groups

G ade 3 Mainly fine granular haemosiderin but also large granules in most cell groups

Grade 4 Stainable iron very abundant in virtually all cell groups, to a large extent in the form of gross granu es and agregations of haemosiderin

The histological needle biopsy sections were examined at 600 magnification (Fig ?) The following grading was used (only parenchymal liver cell iron was graded)

Grade 0 No stainable iron or only isolated fine granules in the whole preparation

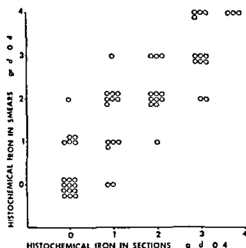


Fig 3 Grade of histochemical iron in fine needle aspiration biopsy smears as related to grade of histochemical iron in needle biopsy sections

Grade 1 Fine granular haemosiderin in single cells or in small scattered cell groups
Grade 2 Fine granular haemosiderin in a few or several cells present in most lobules
Grade 3 Mainly fine granular haemosiderin but also large granules present in the periphery of all lobules
Grade 4 Stainable iron very abundant in the major part of the lobule to a large extent in the form of gross granules and aggregations of haemosiderin

RESULTS

Relation of histochemical grade of iron in smears to histochemical grade of iron in sections (Fig 3)

The relation between the histochemical grade of iron in smears and in sections was studied in the whole series As Fig 3 shows there was a good *agreement between the two methods In only two* patients did the difference between the methods exceed one grade Higher gradings in smears compared with sections were more often encountered (21/59) than the reverse (5/59)

Relation between chemical iron (with protein as reference basis) and histochemical iron

The relation between chemical iron in thick needle biopsy specimens and histochemical iron in smears and in sections was studied in 55 patients

The relation between chemical iron and histochemical iron in *fine needle biopsy smears* is shown in Table I and Fig 4 The mean non haemin iron concentration increased with higher histochemical grading but there was a wide range of iron concentrations in each histochemical grade The mean iron concentration of grade 0 was not significantly lower than that of grade 1 ($0.10 > p > 0.05$) but significantly lower than that of grade 2 ($p < 0.005$) and significantly lower than the mean iron concentration of those with grade 1 and 2 ($p < 0.005$) There was no statistically significant difference between those with grade 1 and those with grade 2 but the difference between those with grade 2 and those with grade 3 was significant ($p < 0.005$)

The relation between chemical iron and histochemical iron in *histological needle biopsy sections* is shown in Table I and Fig 5 As in histochemical iron estimate of smears there was a high degree of overlapping of the non haemin iron values of the different histochemical gradings The mean non haemin iron concentration of those with grade 0 however was significantly lower than that of those with grade 1 ($p < 0.05$) The difference between the mean iron concentration of those with grade 1 and those with grade 2 was not significant The *overlapping between the non haemin iron values* of grade 2 and grade 3 was not so marked as in smears and there was no overlap between the

Table I *Non haemin iron concentrations with protein as reference related to histochemical iron in smears and in sections*

Grade of histochemical iron	No	Smears (mg/100 g protein) Mean ± S.E. of mean	Range	No	Sections (mg/100 g protein) Mean ± S.E. of mean	Range
0	14	103 4 ± 11 8	34–168	17	110 2 ± 11 1	34–213
1	10	154 2 ± 24 9	90–328	13	167 4 ± 17 2	74–263
2	16	170 1 ± 15 8	74–296	12	182 9 ± 20 5	82–328
3	9	264 3 ± 27 7	169–411	10	361 3 ± 39 0	209–580
4	7	1131 ± 35 7	298–2845	3	2079	1446–2845

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FOUR YEARS FOLLOW UP OF ASYMPTOMATIC ISOLATED PROTEINURIA DIAGNOSED IN A GENERAL HEALTH SURVEY

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Abstract The present work comprises a follow-up study on average four years after of 128 cases displaying isolated proteinuria of persistent (31 cases) intermittent (55 cases) or transient type (4? cases) in a general health survey The check-up examination included a screening with questionnaire and analysis of morning urine sent in and also a clinical examination of cases displaying pathological urine findings or suspect case histories of urinary tract disease in the screening In the clinical examination 89 individuals took part (?0 cases with persistent, 45 with intermittent and 24 with transient proteinuria) Of these 89 cases only four (4.5%) had proteinuria in all three urine examinations which were performed All these four cases had persistent proteinuria at the primary examination Sixty-eight cases (76%) did not have proteinuria on any occasion during the follow up six of whom had persistent proteinuria at the primary examination. Only 11 (12%) of the 89 clinically investigated displayed signs of urinary tract disease Seven of these 11 cases with urinary tract disease belonged to the group with persistent proteinuria at the primary examination

At health examinations it is not altogether uncommon to find proteinuria without other signs of urinary tract disease In general greater significance is attached to an isolated proteinuria of this kind if it is constant than if it is intermittent (e g orthostatic) or transient In recent years however it has been found at biopsies that even with the two latter forms of proteinuria pathological changes may be found in the kidney tissues

Discovery of proteinuria often leads to costly extensive investigations with accompanying medical checks which may involve mental strain for the patient It would therefore clearly be of great significance if one could prognostically evaluate an "isolated proteinuria With the object of getting some idea of the prognosis for different forms of isolated proteinuria a follow up study was therefore undertaken after three to five years The subjects were individuals who at a general health examination were found to have proteinuria without other clinical signs of urinary tract disease

MATERIAL AND METHODS

During 1962–1964 the Malmohus County Council carried out a general health examination which from the nephrological viewpoint, primarily consisted of a screening for proteinuria with Albustix Cases in which the results appeared to be positive were subjected to policlinic investigation comprising a general medical examination urine tests (Albustix, Heller s nitric acid ring test, Addis quantitative sediment bacteria culture) ESR, Hb and in most cases determination of serum creatinine and intravenous urography Kidney biopsy however was not performed—an investigation which would have been extremely valuable In 1967 a follow up was made of the 128 cases which apart from proteinuria had no other signs of urinary tract disease at the above mentioned health examination

At the screening in 1962–64 only the Albustix test was performed proteinuria being thereby defined as ⩾2+ On the other hand both Albustix and Heller s nitric acid ring test were used at the policlinic investigation in 196 –64 and at the check-up in 1967 Proteinuria was at that time considered to exist with ⩾ + Albustix plus a positive Heller s nitric acid ring test

The 1 8 follow-up cases were divided into three groups from the results of the proteinuria tests which were carried out at the primary examination in 1962–64 in day urine in conjunction with the screening in the field, in day and night urine respectively at the subsequent examinations (Table I)

1 Transient proteinuria (proteinuria only at the time of screening) 42 cases, ?3 women and 19 men. Mean age 26 years (range 16–60 years)

Table I *Age distribution of the screened individuals*

Age group	Transient proteinuria Male	Female	Total	Intermittent proteinuria Male	Female	Total	Persistent proteinuria Male	Female	Total
10–19	8	8	16	16	10	26	5	9	14
20–29	4	9	13	10	6	16	5	—	5
30–39	4	3	7	2	3	5	1	1	2
40–49	1	1	2	7	1	8	5	2	7
50–59	2	1	3	—	—	—	2	—	2
60–69	—	1	1	—	—	—	1	—	1
Total	19	23	42	35	0	55	19	12	31

Intermittent proteinuria (proteinuria at the screening and in day or night urine at the clinical examination) 55 cases 20 women and 35 men Mean age 24 years (range 15–46 years)

3 Persistent proteinuria (proteinuria at all three examinations) 31 cases 12 women and 19 men Mean age 27 years (range 16–60 years)

As can be seen from Table I the age distribution was similar in the different groups

The first part of the check up was done by post The patients answered a questionnaire about urinary tract diseases and at the same time they sent in a sample of urine morning urine to which was added four drops of 36% formalin (A) The following tests were made on e urine sent in Albustix, Heller s nitric acid ring test, talase test Clinistix, Haemastix, nitrite test and micro opic examination of sediment Individuals who exhibited pathological urine findings and/or had suspect case histories of urinary tract disease were called to a clinical examination which took place between 3–86 days later (on average 17 days)

At the clinical examination the same tests were made as previously partly on night urine which was tes ed for Addis quantitative sediment (B) and partly on day urine passed at the time of the examination after washing the outer genitalia with sodium chloride solution (C) A portion of the latter urine sample was cooled to +4 C and sent for semiquantitative culture to the central bacteriological laboratory of the hospital (Head R Grubb M D) In 39 cases an X ray examination was mad comprising a survey of the urinary tract and intravenous urography Thirty four of these cases had also been investigated at the primary examination in 1962–64 when a total of 121 out of 178 proteinuria cas s had been X rayed

Unfortunately at the follow up examination it was again not practically possible to perform kidney biopsies

RESULTS

Screening

A *Analysis of morning urine sent in*

1 *Proteinuria* As can be seen from Table II proteinuria as defined earlier was found in a total of 16 cases (12 5%) Thirteen (41 9%) of the 31 cases which in 1962–64 had what was designated persistent proteinuria had proteinuria in the urine sp cimen sent in 1967 Corresponding figures for cases with transient proteinuria were three (7 1%) out of 42 while none of the 55 cases with intermittent proteinuria displayed proteinuria in this urine sample

2 *Pyuria* (> 5 WBC/HPF) occurred in a total of five cases (3 9%) The frequency was the same in the different proteinuria groups

3 *Haematuria* defined as >2+ Haemastix was found in altogether three cases (2 3%) one case in each proteinuria group

Table II *Results of the 1967 screening examination*

Group of proteinuria	No examined	Proteinuria No		Pyuria No		Hematuria No	%
Transient	42	3	7 1		4 7	1	2 4
Intermittent	55	—	—	2	3 6	1	1 8
Persistent	31	13	41 9	1	3 2	1	3
Total	1 8	16	12.5	5	3 9	3	2 3

Table III *Age distribution of the clinically examined individuals*

Age group	Transient proteinuria			Intermittent proteinuria			Persistent proteinuria		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
10-19	7	4	11	13	9	2_	2	7	9
0-29	3	5	8	8	4	12	4	—	4
30-39	1	—	1	1	3	4	1	1	2
40-49	1	—	1	6	1	7	1	1	2
50-59	2	1	3	—	—	—	2	—	2
60-69	—	—	—	—	—	—	1	—	1
Total	14	10	24	28	17	45	11	9	_0

Clinical Examination

Eighty nine (69%) of the 128 cases examined by post underwent a more thorough policlinical examination (see Table III for age and sex distribution) They were called on very liberal grounds such as case history details of dysuria pollakiuria cloudy evil smelling urine etc (34 cases) or if the screening examination of the urine showed Albustix ≥1+ Haemastix ≥1+ microscopic bacteriuria catalase positive etc (35 cases) In twenty cases there were both suspect case histories and "pathological" urine samples There were no noteworthy differences concerning the motives for the clinical examination between the different proteinuria groups

B *Analysis of night urine brought with patient*

1 *Proteinuria* Nine cases (10 1%) displayed proteinuria in the night urine they brought with them Seven of these cases belonged to the group with persistent proteinuria and two to the group with intermittent proteinuria which gave frequency figures of 35 0 and 4 4% respectively None in the group with transient proteinuria displayed proteinuria in this sample (See Table IV)

Quantitative sediment after Addis

2 *Pyuria* (>4 mill WBC/12 h) In all 20 cases (22 5%) displayed pyuria Distribution among the different groups showed a moderate percental predominance for persistent proteinuria as opposed to the other two groups 30 0% against 22 2 and 16 7% respectively for intermittent and transient

3 *Haematuria* (>1 5 mill RBC/12 h) existed in a total of four (4 5) of the 89 cases examined Three of these cases belonged to the group persistent proteinuria, and one case to the group transient proteinuria

C *Analysis of day urine*

1 *Proteinuria* Altogether 12 cases (13.5%) displayed proteinuria in the day urine which was passed at the time of the clinical examination The frequency in the different proteinuria groups persistent intermittent and transient was 30 0 6 7 and 12 5% respectively

2 *Quantitative urine culture* More than 10^5 bact/ml urine were found in four cases altogether (4 5% E. coli in all cases) two cases with intermittent and two with persistent proteinuria three women and one man (See Table V)

Table IV *Results of urinalysis at clinical examination*

Urine collected during night (Addis count)

Group of proteinuria	No examined	Proteinuria		>4 mill. WBC/12 h		>1 5 mill RBC 12 h	
		No	%	No	%	No	%
Transient	_4	–	—	4	16 7	1	4 2
Intermittent	45	2	4 4	10	_2	–	—
Persistent	20	7	35 0	6	30 0	3	15 0
Total	89	9	10 1	_0	2_ 5	4	4 5

Table V *Results of analysis of freshly voided urine at the clinical examination*

Group of proteinuria	No examined	Proteinuria No		> 10^5 bact/ml urine No	%
Transient	24	3	12 5	-	—
Intermittent	45	3	6 7	2	4 4
Persistent	20	6	30 0	2	10 0
Total	89	12	13 5	4	4 5

D *Blood analyses*

1 *Serum creatinine* Two of the cases with persistent proteinuria had increased serum creatinine values (1 3 and 1 7 mg% respectively) All cases with intermittent or transient proteinuria displayed normal values

2 *ESR* Of the 89 examined 15 (16 9%) had an ESR in excess of 15 mm/1 h and three more than 30 mm/1 h

3 *Antistreptolysin titre* Thirty seven (41 6%) of those examined had an antistreptolysin titre of more than 140 IU (the normal limit at this bacteriological laboratory) and 12 cases (13 5%) more than 500 IU These latter cases were distributed equally among the proteinuria groups ich gave a frequency of 20% for persistent nuria 9% for intermittent and 17% for , proteinuria (See Table VI)

E *Other investigations*

1 *Blood pressure* Two cases of hypertension were recorded in the group with persistent proteinuria but no significant difference was found between the different groups for either diastolic or systolic pressure

2 *X ray examination* At the health survey in 1962–1964 X ray examinations (urinary tract survey and intravenous urography) were made in 121 of the 128 cases which initially took part in the follow up study in 1967 with normal findings as the result In 1967 39 cases were X rayed 34 of which had also been investigated in 1962–64 No pathological findings were recorded at the 1967 examination either

F *Persistent intermittent and transient proteinuria at the 1967 examination*

If the cases which took part in the clinical examination in 1967 were grouped according to the number of occasions they displayed proteinuria (transient proteinuria 1 occasion intermittent 2 occasions persistent 3 occasions) it was found that only four cases (4 5%) of the 89 examined had persistent proteinuria three (3 4%) intermittent and 14 (15 7%) transient proteinuria The remaining 68 cases (76 4%) did not have proteinuria (according to our definition) at any time The four cases which had persistent proteinuria at the follow up examination belonged to the 20 cases which in 1962–64 were classified as persistent proteinuria Of the remaining 16 cases in this group six did not have proteinuria at any one of the three examinations in 1967 while eight had transient and two had intermittent proteinuria Thus only one fifth of the cases which at the first examination displayed proteinuria at all the examinations still had constant proteinuria 3–5 years later and of the 69 cases

Table VI *Results of blood analysis at the clinical examination*

Group of proteinuria	No examined	ESR > 15 mm/1 h No		Creatinine s > 1 2 mg% No	%	Antistreptolysin titre > 140 IU No	
Transient	24	4	16 7		—	10	41 7
Intermittent	45	6	13 3	-	—	18	40 0
Persistent	20	5	25 0		10 0	9	45 0
Total	89	15	16 9	•	-2	37	41 6

Table VII *Number of individuals with no proteinuria and with proteinuria on one two or three occasions at the clinical examination*

Tests in 1967	Frequency of the different forms of proteinuria 196 –1964			
	Transient proteinuria	Intermittent proteinuria	Persistent proteinuria	Total
No proteinuria	21	41	6	68
Proteinuria once	3	3	8	14
Proteinuria twice	—	1	2	3
Proteinuria thrice	—	—	4	4
Total	24	45	20	89

which in 1962–64 displayed transient or intermittent proteinuria none had become persistent at the follow up study Sixty two of these cases (89 9%) did not have proteinuria at any time during the follow up

G *Clinical assessment*

Seventy seven (87) of the 89 cases subjected to closer examination were considered to show no signs of urinary tract disease When taking into account the initial distribution into the different proteinuria groups it was found that 65 % of the persistent proteinuria group 93% of the intermittent group and 92 % of the transient proteinuria group were considered to be healthy In the persistent proteinuria group were noted two certain and one suspected case of urinary tract infection one case of chronic pyelonephritis two cases of hypertension and one additional case who had had nephrolithiasis between the examinations

Two cases of urinary tract infection and one case with a history of repeated attacks of cystopyelitis were recorded in the intermittent proteinuria group

Among the cases with transient proteinuria there was one with isolated haematuria and one case of gonorrhoea This latter case would not appear to be of interest in this connection

The four cases displaying persistent proteinuria both in 1962–64 and in 1967 were assessed as being clinically healthy apart from the proteinuria and they were not subjected to any form of therapy

H *Other points of interest*

It is worth noting that of 72 cases who were advised to have regular medical checks on the basis of the findings at the examination in 1962–64 only 42 (68%) had had these checks The lowest figure was recorded in the intermittent proteinuria group in which only 20 (57) out of 35 who had been recommended to have checks had followed this advice

DISCUSSION

It is scarcely possible to conceive of a single definition of the term proteinuria (15) since among other reasons the protein excretion of normal persons varies within wide limits Thus vigorous physical activity even in persons with healthy kidneys leads to such a great rise in excretion of serum protein in the urine that the common routine tests for proteinuria may be positive (16 20 21 30) Account must therefore also be taken of the test conditions when judging an isolated proteinuria test There is also conflicting information about the normal daily excretion of protein in the urine under standardised conditions (7 16 22 29)

In recent years Albustix because of its simple procedure has to an ever increasing extent replaced the previously used precipitating reactions (Heller's nitric acid ring test and the sulphosalicylic acid test) There is however disagreement in the literature as to the value of the test (1 4 5 6 12 15 17 25 27 28) With the object of increasing the certainty of proteinuria diagnosis therefore we did not in the present investigation consider proteinuria to be present until both the Albustix reaction and Heller's nitric acid ring test were positive During the screening in 1962–64 however only Albustix was used For this reason the diagnosis transient proteinuria

which was based solely upon this test must be regarded as uncertain in this connection It must also be pointed out that had the primary examination and the follow up study comprised more than three examinations there would undoubtedly have been a certain amount of regrouping among the proteinuria types

On this account and since previously published articles often lacked a clear definition of the different concepts of proteinuria it is difficult to make any direct comparisons between the studies presented here and earlier investigations King found in his follow up study that what he called persistent proteinuria seldom disappeared or became intermittent (9) In other investigations however it was found that non persistent proteinuria could completely disappear but could also sometimes become persistent (2 9 10 18 19 26) The results of the present investigation also lend weight to the argument that inconstant proteinuria may disappear for we found that $^4/_5$ of the cases which at the primary examination had what is here designated transient or intermittent proteinuria after four years showed negative proteinuria findings in three consecutive examinations The remainder of the cases in both these groups still had proteinuria of type intermittent or transient Thus none of these cases had at that time become persistent Of those cases which from the given definition were judged to have persistent proteinuria at the primary examination only 20% still had constant proteinuria at the follow up examination while fully 30% showed negative findings throughout This could indicate that a number of the cases which were judged to be persistent at the primary examination would actually have proved to be intermittent if more examinations had been performed at that time

The frequency of pyuria at the screening in the follow up study was only 3 9% but in quantitative sediment at the clinical examination fully 22 5% had pyuria This figure which perhaps seems rather high should however be compared with the frequency of pyuria (33 5%) which was obtained by the same method at another examination of subjectively healthy women (11)

The frequency of haematuria too (2 3% in screening and 4 5% at follow up) is not different from the frequency obtained in the above mentioned study (4 4%) As a comparison Lecocq s follow up of fixed orthostatic proteinuria may be mentioned in which after five years he found only one case of haematuria among 61 cases investigated (14) Considering that in the literature the frequency of asymptomatic bacteriuria among women is given as 2 2–6 6% (8) and that the bacteriological diagnosis in the present work is based on only one culture the bacteriuria frequency obtained should not be markedly different from the frequency in a normal" material

The frequency of cases with increased antistreptolysin titre seems to be remarkably high even taking into account that the investigation was made in the autumn Since we do not have access to any control material the frequency figures obtained cannot be evaluated

Of the two cases with persistent proteinuria which displayed increased serum creatinine values of 1 3 and 1 7 mg% respectively one case had normal creatinine clearance and the other had hypertension with nephrosclerosis

King (10) and Phillippi et al (19) in follow up investigations of young men with persistent proteinuria recorded diastolic hypertension in 57 and 34% respectively Concerning orthostatic proteinuria King reported a rise in diastolic blood pressure in 15% while Lecocq could not verify this in his work (14) In the present study we found two cases of hypertension in the persistent proteinuria group but no difference in the mean values for systolic and diastolic blood pressure between the different proteinuria groups

In the above mentioned investigation King found that of cases still displaying constant proteinuria at the follow up 53% clinically had chronic pyelonephritis (10) while Phillippi et al who in his follow up study of persistent proteinuria performed kidney biopsies found light microscopic glomerular changes in 74% (19) The results of the investigations mentioned are of course difficult to compare with our own A number of the cases which as a result of the proteinuria findings of 1962–64 were called persistent would for example certainly have been intermittent as was mentioned earlier The latter situation is in all probability part of the explanation why fully 65% of the cases with persistent proteinuria in 1962–64 did not display clinical signs of urinary tract disease at the check up in 1967 On the other hand the four cases

which still had persistent proteinuria in 1967 did not show signs of kidney disease either

Concerning non persistent forms of proteinuria previous follow up studies which in a number of cases included kidney biopsy have revealed both overt and sub-clinical kidney diseases and also a number of cases of metabolic disturbance (14 17 23 24) In the present check up on the cases displaying transient or intermittent proteinuria at the primary examination in 1962–64 urinary tract disease was found in 6° a frequency which does no seem to differ from that in a "normal material

To sum up it may be said that for this four year follow up study of cases with different forms of isolated proteinuria the results con cerning pathological findings were strikingly modest It must be pointed out however that a more extensive examination including kidney biopsy would in all certainty have revealed an additional number of cases with kidney diseases Bearing in mind the higher mortality for dif ferent forms of isolated proteinuria compared with normal material which has been demon strated by long term observations further follow up studies of the material presented here would be of great interest (3 13) The results of the present study indicate that in cases of transient or intermittent isolated proteinuria there is no medical motivation for too frequent checks which may lead to the patient becoming psychically invalided The observation that only $^{?}/_{3}$ of the individuals who had been recommended to have continued medical checks at the primary examina tion in 1962–64 had taken this advice is remark able This serious situation which reduces the value of general health surveys should be noted when planning the organisation of similar exami na ions

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Group C renal autotransplantation with uretero-ureteral anastomosis followed by contralateral nephrectomy

Conventional technique was used for performance of the nephrectomy in Group A.

The autotransplantation in Group B was commenced by nephrectomy performed so that the renal artery and vein were severed close to the inferior vena cava and aorta, respectively. The ureter was severed while maintaining its vascular supply and ligated at a distance of 10–15 cm from the kidney. After removal the kidney was treated as described in the section dealing with preparation of the kidney. The kidney was reimplanted by end-to-end anastomosis of the renal artery and the external iliac artery and by end-to-side anastomosis of the renal vein and the external iliac vein, generally on the right side, using 6-0 silk on an atraumatic needle. Re-establishment of the urinary tract was ensured after the ureter was spatulated on the distal 1 cm, by suturing the ureter into the lumen of the bladder and then sewing the wall of the bladder to the adventitia of the ureter. Ureteral anastomosis was done with 5-0 atraumatic catgut. Conventional technique was used for the contralateral nephrectomy.

The autotransplantation in Group C was performed as described for Group B, except that the urinary tract was re-established by means of a uretero-ureteral anastomosis. When placing this, the two ends of the ureter were spatulated before suturing with 5-0 atraumatic catgut. Contralateral nephrectomy was performed by the conventional method.

At the end of the operation ½ million I.U. benzylpenicillin sodium NFN (Penicillin Leo®) and 1 g streptoduocinum NFN (Duo-Streptomycin Novo Novojecti®) were administered into the peritoneal cavity. The abdominal wall was closed in several layers with interrupted sutures of silk. On each of the first four days after the operation the pigs were given 16 000 I.U. per kg benzylpenicillinum NFN and 70 mg per kg dihydrostreptomycini sulfas NFN (Streptopenprokain Rosco vet®). The sutures were removed ten days after the operation.

Preparation of the Kidney

After removal the kidney was immediately perfused with 500 ml cold (4 C) low molecular weight dextran (average MW 40 000) (10% Rheomacrodex with sodium chloride®) containing 2500 I.U. heparinum NFN (Heparin® Leo) and 1 g procaini chloridum NFN administered from a flask at a height of about 130 cm. The perfusion time was from 10 to 20 min. The temperature 15 min inside the kidney was measured in three cases and at the end of the perfusion the average temperature was 13 C (thermistors made by E. L. Laboratories, Copenhagen). Reimplantation was started immediately after cessation of the perfusion, and during stitching the kidney was not cooled externally. Recirculation with blood commenced after 30 to 50 min and the total ischaemic time thus varied from 40 to 70 min.

Postoperative Studies

Blood analyses. During the first week after the operation, blood specimens for the determination of pH, haematocrit and concentrations of creatinine, urea, sodium, potassium and chloride in plasma were taken every other day and subsequently once a week for the following three months.

Kidney function. Simultaneous determinations were made of the clearance of inulin, endogenous creatinine, urea, and para-amino-hippuric acid (PAH) and of the excretion percentages of water, sodium, potassium and chloride. The experiments were made in unanaesthetized animals. The first experiment was done ten days after the operation and the following experiments every third week. Each experiment consisted of at least three periods of 10 min and the last experiment concluded with three periods for the determination of the maximal excretion (Tm) of PAH. For details of the test substances, clearance technique and methods of calculation see Gyrd-Hansen (9).

Analytical methods. Inulin: Brun (3); endogenous creatinine: Bonsnes and Taussky (1); PAH: Bratton and Marshall (2); urea: Conway (4); chloride: Schales and Schales (21). Potassium and sodium were determined by flame photometry (Beckman Direct Reading Flame Photometer). All the plasma concentrations found were calculated to correspond to the value in the water phase of the plasma, being assumed to be 93%. The pH was determined by a microglass electrode (Radiometer) and the haematocrit values by a microhaematocrit tube after centrifugation in a MSE Micro Haematocrit Centrifuge for 15 min at 4500 rpm (2,000 × g).

Postmortem examination. After the last clearance experiment the animals were killed by shooting and bled, and a postmortem examination performed immediately. The kidneys were weighed and tissue was removed for histological examination and fixed in buffered formalin. Paraffin wax sections were stained with iron haematoxylin-van Gieson, and the periodic acid Schiff reaction was carried out according to McManus and Mowry (15).

RESULTS

Operation was performed on 17 pigs, i.e. five in each of Groups A and C, and seven in Group B. Two of the latter died about a month after the operation as a result of ureteral stenosis with the development of hydronephrosis and uraemia and are therefore omitted from the Group B results.

Concentrations of Creatinine, Urea, Sodium, Potassium and Chloride in Plasma

Fig. 1 shows the average concentrations of creatinine (µg/ml) in plasma in Groups A, B and C during 105 days after the operation.

It will be seen that in Groups B and C the creatinine concentration in the plasma of pigs increased to higher levels than in the Group A animals during the first six days after the operation. This difference was statistically significant

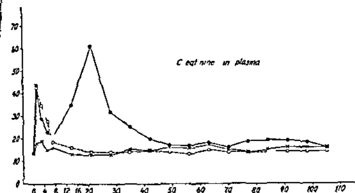


Fig 1 Concentration of creatinine in plasma 0–105 days after operation *Ordinate* creatinine in plasma μg/ml *Abscissa* days after operation ×—× Group A ●—● Group B ○--○ Group C

on the 1st day ($p<0.001$) On the 7th day the creatinine concentration in plasma was not significantly different in the three groups During the remainder of the observation period there was only a slight difference between the creatinine concentration in plasma in Groups A and C ($p>0.10$) The creatinine concentration in plasma of the Group B pigs increased from the 7th day and reached its maximum on the 21st day However the standard deviation in Group B was so great that comparison with Group A on the 21st day showed no significant difference ($0.05<p<0.1$) From the 50th day the creatinine concentration in plasma in Group B was only slightly higher than in Group A ($p>0.025$)

Fig 2 shows the concentration of urea in plasma of the pigs in the three groups It will be seen that the urea content varied during the experimental period exactly in parallel to the creatinine content (Fig 1) The concentration of sodium potassium and chloride in plasma was constant throughout the experimental period and was identical in the three groups viz 143 mEq/l (128–164) 4 4 mEq/l (3 3–5 3) and 109 mEq/l (102–115) respectively

The average haematocrit varied during the experimental period from 33 to 42 The lowest values were observed in Group B during the first four weeks after the operation (Tables I II and III)

The pH of the blood was quite constant during the experimental period and was the same for the three groups with an average of 7.39 ± 0.06

Clearance of Inulin Endogenous Creatinine Urea and PAH

Tables I II and III show the average values for Groups A B and C In Group A the clearances as well as total renal plasma flow (RPF_{total}) and total renal blood flow (RBF_{total}) remained constant during the experimental period

In Group B clearance as well as RPF_{total} and

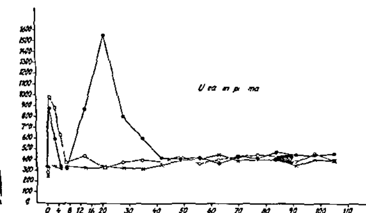


Fig 2 Concentration of urea in plasma 0–105 days after operation. *Ordinate* urea in plasma μg/ml *Abscissa* days after operation ×—× Group A ●—● Group B ○--○ Group C

Table I *Average renal clearances in five pigs 10 to 94 days after unilateral nephrectomy Group A*

The bracketed figures indicate minimal and maximal values of single experiments

Days after nephrectomy	Body weight (kg)	Haematocrit (%)	Diuresis (ml/min)	Clearance: Inulin (ml/min/10 kg b.wt.)	Clearance: Endogen. creatinine (ml/min/10 kg b.wt.)	Clearance: Urea (ml/min/10 kg b.wt.)	Clearance: PAH (ml/min/10 kg b.wt.)
10	[illegible]	38 (36–41)	0.8 (0.[illegible]–0.9)	16 (14–19)	16 (12–20)	8 (7–10)	52 (4[illegible]
31	75 (70–79)	40 (37–41)	1.3 (1.1–1.5)	17 (15–19)	18 (16–20)	9 (6–11)	53 (45–49[illegible]
5[illegible]	90 (83–9[illegible])	41 (40–43)	1.7 (1.4–2.0)	17 (15–19)	17 (16–19)	9 (5–10)	55 (4[illegible]
73	106 (100–112)	39 (35–43)	2.2 (1.8–2.6)	17 (15–20)	17 (16–19)	10 (9–1[illegible])	55 (4[illegible]
94	117 (111–126)	42 (39–44)	2.2 (1.5–3.0)	17 (15–17)	17 (16–19)	10 (9–11)	57 (50–[illegible]

Table II *Average renal clearances in five pigs 10 to 94 days after bilateral nephrectomy and unilateral autotransplantation Uretero-vesical anastomosis Group B*

The bracketed figures indicate minimal and maximal values of single experiments

Days after transplantation	Body weight (kg)	Haematocrit (%)	Diuresis (ml/min)	Clearance: Inulin (ml/min/10 kg b.wt.)	Clearance: Endogen. creatinine (ml/min/10 kg b.wt.)	Clearance: Urea (ml/min/10 kg b.wt.)	Clearance: PAH (ml/min/10 kg b.wt.)
10	60 (4[illegible]–67)	35 (27–42)	1.9 (0.[illegible]–5.4)	10 (7–15)	12 (7–16)	6 (3–9)	[illegible]
[illegible]1	67 (57–[illegible]6)	35 (33–47)	3.9 (1.3–8.0)	1[illegible] (7–22)	12 (8–21)	7 (4–11)	4[illegible] (2[illegible]
[illegible]	85 (72–96)	38 (34–47)	2.5 (1.9–3.1)	14 (11–19)	17 (11–21)	9 (7–12)	41 (30–[illegible]
[illegible]3	95 (85–10[illegible])	41 (39–43)	3.1 (1.6–6.4)	15 (1[illegible]–19)	18 (13–22)	9 (7–10)	5[illegible] (4[illegible]
94	113 ([illegible]–122)	40 (38–43)	3.4 (1.9–5.3)	15 (11–20)	15 (11–20)	9 (7–13)	40 (3[illegible]

Table III *Average renal clearances in five pigs 10 to 94 days after bilateral nephrectomy and unilateral autotransplantation Uretero-ureteral anastomosis Group C*

The bracketed figures indicate minimal and maximal values of single experiments

Days after transplantation	Body weight (kg)	Haematocrit (%)	Diuresis (ml/min)	Clearance: Inulin (ml/min/10 kg b.wt.)	Clearance: Endogen. creatinine (ml/min/10 kg b.wt.)	Clearance: Urea (ml/min/10 kg b.wt.)	Clearance: PAH (ml/min/10 kg b.wt.)
10	54 (43–64)	33 (24–39)	2.0 (0.8–[illegible]3)	13 (7–19)	15 (9–22)	7 (4–10)	47 ([illegible]
31	72 (65–86)	38 (35–41)	1.7 (1.2–2.3)	19 (17–23)	19 (18–20)	11 (10–11)	69 (6[illegible]
52	85 (77–97)	38 (34–47)	1.7 (0.9–[illegible]2)	19 (18–21)	20 (18–[illegible]1)	10 (9–17)	70 (4[illegible]
[illegible]3	105 (100–112)	40 (38–43)	3.0 (2.5–3.9)	18 (17–19)	20 (19–21)	1[illegible] (11–13)	6[illegible] (4[illegible]
94	120 (111–124)	40 (38–44)	[illegible]6 (2.3–2.8)	18 (17–19)	18 (16–19)	10 (9–13)	67 (5[illegible]

RBF_{total} increased until 73 days after the transplantation and then became stabilized.

In Group C clearances as well as RPF_{total} and RBF_{total} increased until the 31st day and then became stabilized. In calculating the total renal plasma flow an extraction percentage of 87 was used, as found by Gyrd-Hansen (9) in normal pigs.

Since the experimental animals were immediately after the last clearance experiment it was possible to state the clearances both [illegible] 10 kg body weight and per 100 g kidney [illegible] for the final measurements. The average results for Groups A, B and C are shown in Table IV which also gives the corresponding values for normal pigs (9).

Total renal plasma flow (ml min 10 kg b wt.)	Total renal blood flow (ml m n 10 kg b wt)	Clearance ratios		
		Cr In	Urea, In	Filtration fraction In PAH
65 (60–75)	105 (94–121)	1 0	0 5	0 31
66 (56–86)	109 (89–143)	1 1	0.5	0.31
69 (60–76)	117 (105–131)	1 0	0 5	0.3_
69 (59–92)	114 (95–156)	1 0	0 6	0.32
72 (6 101)	123 (107 171)	1 0	0 6	0.34

Total renal plasma flow (ml min 10 kg b wt.)	Total renal blood flow (ml min/10 kg b wt.)	Clearance ratios		
		Cr In	Urea, In	Filtration fraction In PAH
41 (26–63)	63 (36–98)	1 _	0 6	0 31
53 (33–94)	84 (47–16)	1 0	0 6	0.29
54 (38–83)	88 (63–143)	1.2	0 6	0 31
65 (50–93)	111 (84–163)	1.2	0 6	0.29
63 (41 90)	105 (67–150)	1 0	0 6	0 30

Total renal plasma flow (ml m n, 10 kg b wt.)	Total renal blood flow (ml m n 10 kg b wt)	Clearance ratios		
		Cr In	Urea/In	Filtration fraction In PAH
59 (31 106)	106 (47–140)	1 2	0 5	0 _8
86 (76–101)	138 (129–158)	1 0	0 6	0.28
88 (76–96)	142 (115–166)	1 1	0 5	0 8
78 (60–89)	130 (97–143)	1 1	0 7	0.29
84 (69 99)	140 (119–160)	1 0	0 6	0 27

Maximal Tubular Excretion of PAH

The Tm of PAH was determined in the last experiment and the results are shown in Table V for each pig separately together with the plasma concentrations of PAH on which the Tm determinations were made The Tm values are given both per 10 kg body weight and per 100 g kidney tissue

Excretion of Water Sodium Potassium and Chloride

The excretions of water sodium, potassium and chloride for pigs in Groups A, B and C are shown in Tables VI VII and VIII These values were quite constant from the 10th to the 94th day in each group but for both water and electrolytes the values were higher in Groups B and C than in Group A

Postmortem Examination

Macroscopical findings

In the unilaterally nephrectomized pigs (Group A) the kidney had normal colour The kidney weights are shown in Table IX the average weight was 0 26% of the body weight

In Group B the kidneys from pigs 16 26 27 and 28 were normal in colour but firm. In pig 18 one pole of the kidney was completely atrophied and the remaining tissue had a normal colour but was very firm. In pigs 16 18 27 and 28 the papillae were strongly flattened and the pelvis and ureter were greatly distended. There was a swelling at the entry of the ureter into the bladder which caused a severe stenosis of the distal part of the ureter In the kidney from pig 26 the papillae and pelvis were normal and the ureter only slightly distended. There was only a little narrowing around the opening to the bladder The relative kidney weights of the pigs in Group B are shown in Table IX the average weight was 0 32% of the body weight.

As regards Group C the kidneys were of normal colour and were slightly firm The papillae and pelvis were normal There was a slight narrowing at the ureter anastomosis, and proximally to the anastomosis the ureter was only slightly dilated. The relative kidney weights are shown in Table IX the average weight was 0 37% of the body weight

In all the pigs both the arterial and venous vascular anastomosis is healed without any sign of stenosis or thrombus formation.

Microscopical findings

Apart from slight interstitial fibrosis in the kidney from pig 23 the kidneys from the Group A animals were normal

In Group B pig 18 had chronic purulent pyelonephritis comprising about 10% of the kidney

Table IV *Average renal clearances in pigs 94 to 115 days after operation*

	Clearance							
	Inulin		Endogenous creatinine		Urea		PAH	
Group	(ml min/ 10 kg b wt.)	(ml min/ 100 g kidney)	(ml min/ 10 kg b wt.)	(ml min/ 100 g kidney)	(ml min/ 10 kg b wt.)	(ml min/ 100 g kidney)	(ml min/ 10 kg b wt.)	(ml min/ 100 g kidney)
A	16	59	16	61	10	36	56	209
B	14	46	16	49	9	27	54	171
C	18	49	18	48	10	29	67	18[illegible]
Normal pigs	21	56	22	61	12	34	64	174

Gyrd Hansen 1968 (9)

tissue The kidneys from pigs 16 27 and 28 showed focal interstitial nephritis and interstitial fibrosis which was most pronounced around the glomeruli and vessels as well as in the medulla The kidney from pig 26 was normal

In Group C the kidneys from three pigs (nos 30 33 and 39) showed a slight degree of dif fuse interstitial fibrosis There was focal intense scar like connective tissue in the renal cortex in [illegible] 35 while the kidney from pig 37 was normal

DISCUSSION

The concentration of creatinine and urea in plasma (Figs 1 and 2) was within normal limits for the nephrectomized pigs (Group A) during the whole observation period According to Gyrd Hansen (9) the normal values are 7 to 19 μg/ml for creatinine and 240–590 μg/ml for urea In contrast the concentration of creatinine and urea increased immediately after autotransplantation by both techniques and then became normal within a week A similar increase in the urea concentration in plasma has been seen after autotransplantation in dogs (7) and homotransplantation in man (24) The increase in the creatinine and urea concentration in plasma observed from the 2nd to the 7th week in the Group B animals was probably due to transient stenosis at the uretero-vesical anastomosis

Table V *Clearance and maximal tubular excretion (Tm) of para-aminohippuric acid 94 to 115 days after operation*

Group	Pig no	Body weight (kg)	Kidney weight (g)	Plasma concentration of PAH (μg ml)	PAH clearance (ml min 10 kg b wt.)	Inulin clearance (ml min 10 kg b wt)	Tm (mg min 10 kg b wt)	Tm (mg m[illegible] 100 g kidney)
A	[illegible]3	1[illegible]7	[illegible]65	10[illegible]0	6	1	16	6
	25	115	305	1090	31	13	19	[illegible]0
	3[illegible]	140	400	1600	4	11	1	7
	39	111	304	1490	25	12	[illegible]0	73
Average							19	[illegible]
B	[illegible]6	117	395	710	41	11	19	[illegible]
	27	130	450	930	34	14	19	54
	28	136	400	1110	[illegible]8	13	[illegible]0	[illegible]9
Average							19	[illegible]
C	30	118	3[illegible]0	18[illegible]0	[illegible]6	11	[illegible]1	[illegible]0
	33	1[illegible]3	450	930	49	15	33	[illegible]
	35	111	40[illegible]	1000	39	15	[illegible]4	67
	36	1[illegible]4	[illegible]57	1[illegible]40	34	13	6	[illegible]
	37	1[illegible]2	410	11[illegible]5	31	13	1	[illegible]
Average							5	[illegible]

Table VI *Renal excretion of water and electrolytes in five pigs 10 to 94 days after unilateral nephrectomy*

Days after nephrectomy	Body weight (kg)	Diuresis (ml/min)	Inulin clearance (ml/min/ 10 kg b wt)	Excretion			
				Water	Sodium	Potassium	Chloride
10	58	0 8	16	0 83	0 04	11 0	0 38
31	75	1 3	17	0 97	0 0[illegible]	16 5	0.24
52	90	1 7	17	1 11	0 02	22 0	0 24
73	106	2 2	17	1 22	0 11	19 0	0 48
94	117	2 2	17	1 13	0 08	17 7	0 41
Average			17	1 05	0 05	17 2	0 35

Table VII *Renal excretion of water and electrolytes in five pigs 10 to 94 days after bilateral nephrectomy and unilateral autotransplantation Uretero vesical anastomosis*

Days after transplan tation	Body weight (kg)	Diuresis (ml/min)	Inulin clearance (ml/min/ 10 kg b wt)	Excretion			
				Water	Sodium	Potassium	Chloride
10	60	1 9	10	3 18	0 30	29 0	1 10
31	67	3 9	12	4 85	0 48	37 4	1 75
52	85	2 5	14	2 09	0 12	25 0	1 27
73	98	3 1	15	2 11	0 23	24 7	0 93
94	113	3 4	15	2 01	0 21	30 0	1 00
Average			13	2 85	0 27	29 2	1.21

Table VIII *Renal excretion of water and electrolytes in five pigs 10 to 94 days after bilateral nephrectomy and unilateral autotransplantation Uretero ureteral anastomosis*

Days after transplan tat on	Body weight (kg)	Diuresis (ml/min)	Inulin clearance (ml/min/ 10 kg b wt)	Excretion			
				Water	Sodium	Potassium	Chloride
10	54	2 0	13	2.84	0 38	32 0	1 26
31	7[illegible]	1 7	19	1 24	0 05	30 6	0 84
52	85	1 7	19	1 05	0 02	14 2	0 37
73	105	3 0	18	1 59	0 17	30 6	0 89
94	120	2 6	18	1 22	0 22	20 0	0 72
Average			17	1 59	0 17	25 5	0 82

Table IX *Relative kidney weight in pigs 94 to 115 days after operation*

Group	A					B					C				
P g no	20	23	25	32	39	16	18	26	27	28	30	33	35	36	37
Body weight kg	1[illegible]5	127	115	140	111	115	115	117	130	136	118	123	111	124	122
K dney we ght g	353	265	305	400	304	315	375	395	450	400	370	450	402	557	410
Relative kidney weight	0 28	0 21	0 27	0 29	0 26	0 27	0 33	0 34	0 35	0 29	0 31	0 37	0 36	0 45	0 34

Table X. *Comparison between the clearances in normal pigs and in unilaterally nephrectomized pigs*

Days after nephrectomy	p-values for the difference between the clearances of			
	Inulin	Creatinine	Urea	PAH
10	$p<0.001$	$0.01<p<0.02$	$0.001<p<0.005$	$0.05<p<0.10$
31	$p<0.001$	$0.01<p<0.02$	$0.01<p<0.02$	$0.10<p<0.20$
52	$p<0.001$	$0.025<p<0.05$	$0.01<p<0.02$	$0.10<p<0.20$
73	$0.001<p<0.005$	$0.025<p<0.05$	$0.05<p<0.10$	$0.05<p<0.10$
94	$p<0.001$	$0.025<p<0.05$	$0.05<p<0.10$	$0.30<p<0.40$

Comparison of the inulin, endogenous creatinine, urea and PAH clearances in unilaterally nephrectomized pigs with the corresponding clearances in normal pigs with two kidneys (9) shows that the average clearance in the former group during the 94 days of observation was 80–85% of that of normal pigs. The clearance values in nephrectomized dogs (16, 19, 14) and man (23, 6, 18) have been found to be reduced to 60 to 70%. The high compensatory hyperfunction found in the present study is probably due to the fact that the nephrectomy was performed on young animals. Ogden (18) found that the ability to develop compensatory hyperfunction is greatest in young individuals.

Statistical evaluation of the means and standard deviations for nephrectomized and normal pigs gave the p values (t-test) shown in Table X for the differences 10, 31, 52, 73 and 94 days after nephrectomy. In all cases the means are lower in the nephrectomized than in the normal pigs, but a significant *difference (at the 0.01 level)* was found only for inulin clearance and for urea clearance on the 10th day.

The clearance of inulin, endogenous creatinine, urea and PAH were lower in the Group B animals (autotransplantation and uretero-vesical anastomosis) than in nephrectomized pigs. This difference was most pronounced at the examinations on the 10th and 31st days after the operation (Table II), i.e. in the period during which an increased concentration of creatinine in the plasma was observed (Figs. 1 and 2). The results in Group B show large standard deviations, presumably on account of the varying degree of ureteral stenosis. No statistically significant difference (at the 0.01 level) could be demonstrated between Group B and the nephrectomized pigs.

The renal clearances in Group C (autotransplantation and uretero-ureteral anastomosis) in which there were no surgical complications after the operation, were about the same on the 10th day after the operation as those of the nephrectomized pigs. From the 31st day the average clearance values for Group C were higher than those of the nephrectomized animals. This tendency was most marked from the 31st to 73rd day, and the difference on the 52nd day was statistically significant ($p<0.01$) for creatinine and PAH. On the 94th day the difference between the average clearance values for Groups A and C was less ($0.1<p<0.2$). Thus, the renal *clearance in Group C corresponds to that of the* autotransplanted kidneys in dogs (5, 17, 7) and homotransplanted kidneys in man (11, 22, 10, 18) if the organ shows no signs of rejection.

The maximal tubular excretion of PAH both for nephrectomized and autotransplanted pigs (Table VIII) was within the variation found for normal pigs (23 (±6) mg/min/10 kg body weight (9)). Similar results were found in nephrectomized dogs (see 13, 14).

Calculated per 10 kg body weight, the average Tm for Group C was greater than for Group A ($0.025<p<0.05$), while per 100 g kidney tissue it was the same for the two groups ($0.60<p<0.70$). This is in complete agreement with the fact that the relative kidney weights in Group C (0.37) were greater than in Group A (0.26) (Table IX) ($0.001<p<0.005$). The relative kidney weights in Group C were identical with those of normal pigs with two kidneys (0.36) ($0.60<p<0.70$), while they were only about 70% in nephrectomized pigs. This degree of hypertrophy in unilaterally nephrectomized pigs corresponds to the hypertrophy found in unilaterally nephrectomized dogs (20).

Excretion of water was two to five times

higher in Group B pigs than in the nephrectomized animals while in Group C it was one to three times higher The excretion of electrolytes was greater in the autotransplanted than in the nephrectomized pigs which is probably due to the denervating of the transplanted kidney (12) or to damage to the tissue occurring during the ischaemic period

The ureteral stenosis observed and the consequent hydronephrosis together with the histological findings in Group B explain the lower clearances found in that group The slight changes demonstrated at histological examination of the kidneys from Group C have had no effect on the renal clearance These correspond to the changes found by Dempster et al (5) Murray et al (17) and Fontaine (8) in the autotransplanted kidneys of dogs

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RENAL EXCRETION OF LOW AND HIGH MOLECULAR WEIGHT POLYVINYLPYRROLIDONE (PVP) IN PATIENTS WITH PROTEINURIA

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Abstract The renal excretion of two types of PVP with low and high molecular weight (LMW PVP and HMW PVP) has been investigated in normal subjects and in patients with proteinuria due to primary renal disease A significant excretion was found also of HMW PVP

The patients with proteinuria had a slower excretion of PVP than the control subjects During the first hours after injection the quantity of PVP in the urine was much less in the patients This quantity correlated positively with the creatinine clearance

It seems probable that initially the smaller molecules of the PVP sample are excreted and that this excretion is mainly dependent upon the GFR. In a later phase excretion in the patients was on a relatively higher level than in the controls This may be due both to increased glomerular permeability for the larger PVP molecules and to a longer serum half life of PVP

Protein clearances were carried out concurrently which made it possible to divide the patients into a selective and a non selective type of proteinuria The mean ratio LMW PVP/HMW PVP in the non-selective group was different from the selective group and from the control group No difference was found in PVP permeability between the selective group and the control group It is concluded that this PVP ratio may demonstrate differences in glomerular permeability between groups of patients However with the testing material used in this study the overlap is too large for determination of selectivity in individual cases

In recent years glomerular permeability in patients with the nephrotic syndrome has been investigated by clearance studies of serum proteins The selectivity of protein excretion which can be concluded from these clearances is of significant clinical value (3 5 6 7 11 13 14) Apparently there is a correlation between the change in glomerular permeability and the molecular weight distribution of urinary proteins Theoretical objections however to the use of proteins for permeability studies are the varying shape and charge of protein molecules and the possibility of excretion as subunits or complexes

Another approach to glomerular permeability studies can be found in the use of inert macromolecules like dextran (1 4) or polyvinylpyrrolidone (PVP) Recently Hulme and Hardwicke (10) estimated PVP clearances in patients with the nephrotic syndrome as an index of selectivity

The purpose of this study was to investigate the renal excretion of two types of PVP with low and high molecular weight respectively (LMW PVP and HMW PVP) in normal subjects and patients with glomerular proteinuria A ratio of excreted LMW PVP and HMW PVP was assessed and compared with selectivity patterns as estimated by an immunological method

MATERIAL AND METHODS

All patients had proteinuria due to primary renal disease Relevant data concerning renal biopsy diagnosis by light microscopy degree of proteinuria immunologically determined selectivity and creatinine clearance are given in Table I Criteria for histological diagnosis were the same as described in earlier studies from this Department (13 14)

Two series of experiments were arranged In the first series 20 patients (group A nos 1–20 in Table I) were studied during hospitalization Numbers 1–20 in Table I correspond to numbers 1–20 in Table I of the previous article (9) where further laboratory data were presented Simultaneously 19 normal control subjects matched for sex and age and without any symptom of renal disease were studied

In the second series 11 patients (group B) were studied in the Outpatient Department This group comprised both patients already studied in the first series (nos 7 13

Table 1 *Laboratory data renal biopsy diagnosis and urinary PVP excretion in 25 patients with proteinuria due to primary renal disease*

Pat no	Age (y)	Sex	Biopsy diagnosis	Proteinuria (g/24 h)	Selective (S) or non selective (N)	Creatinine clearance	Excreted amounts of PVP in urine (of injected dose)			
							Group A 24 hour excretion			Group B 3 hour excretion
							LMW PVP	HMW-PVP	Ratio LMW-PVP / HMW-PVP	LMW-PVP
1	49	♂	Membr glom nephr	1 1	N	55	21 5	22 2	0 97	
2	57	♂	Focal local glom nephr	0 9	N	69	41 6	22 1	1 88	
3	30	♀	Membr glom nephr	4 3	N	51	4[illegible] 7	19 2	2 22	
4	28	♀	Membr glom nephr	0 7	S	116	42 8	18 3	2 33	
5	66	♂	Focal local glom nephr	3 5	N	54	35 9	14 9	2 41	
6	7	♀	Minimal lesions	0 3	S	120	75 7	25 6	3 96	
7	18	♀	Prolif glom nephr	20 2	N	15	27 7	17 7	1 56	9 6
8	15	♂	Membr glom nephr	10 1	N	104	27 0	16 4	1 65	
9	39	♂	Membr glom nephr	2 9	N	86	30 5	20 5	1 48	
10	42	♀	Membr prolif glom nephr	2 7	N	99	21 0	14 3	1 47	
11	[illegible]5	♀	Membr glom nephr	1 4	N	1[illegible]4	12 0	8 1	1 48	
12	12	♀	Focal local glom nephr	0 7	S	—	35 9	[illegible]0 2	1 78	
13	15	♀	Prolif glom nephr	5 8	N	72	16 8	14 3	1 17	24 7
14	34	♀	Membr glom nephr	4 4	N	77	15 0	10 8	1 38	
15	39	♂	Prolif glom nephr	3 4	N	62	[illegible]6 1	17 4	1 51	26 4
16	73	♀	Membr glom nephr	1 8	S	35	20 8	11 8	1 76	
17	19	♀	Membr glom nephr	8 6	N	140	17 7	15 9	1 32	32 2
18	51	♀	Minimal lesions	1 1	S	92	12.2	15 2	0 80	
19	67	♂	Membr prolif glom nephr	7 7	N	75	14 1	7 8	1 80	13 4
20	37	♂	Minimal lesions	16 3	S	91	41 0	16 5	2.48	22 4
21	16	o	Focal local glom nephr	4 4	N	25				13 0
22	18	♂	Focal local glom nephr	4 7	N	8				19 0
23	21	♂	Prolif glom. nephr	6 8	N	30				16 9
24	24	♀	Lobular glom nephr	8 8	N	60				[illegible]4 4
25	36	+	Membr prolif glom nephr	5 5	N	60				21 0

15 17 19 20) and patients not studied before (nos 21–25) Concurrently with group B 11 control subjects again matched for sex and age were studied

Two types of PVP of a different molecular weight distribution were obtained as pyrogen free sterile isotonic solutions from the Radiochemical Centre Amersham England The mean molecular weights were reported as 40 000 (low molecular weight LMW PVP) and 160 000 (high molecular weight, HMW PVP) respectively Data of analytical ultracentrifuge and gel filtration studies were given in the previous article (9)

For studies in patients of group A and their controls the LMW PVP was labelled with ^{125}I and the HMW PVP with ^{131}I In all cases 25 μc of each type was injected intravenously The two types of PVP were mixed shortly before injection Urine was collected during 24 hours after injection In some patients and controls urine was collected over three consecutive periods on the first day (0–3 3–10 10–24 hours) and in others urinary excretion was also estimated on the second and third day after injection

For studies in patients of group B and their controls another batch of LMW PVP was used which was labelled with ^{131}I Again 25 μc was injected intravenously Urine was collected during the first three hours after injection

In all patients thyroid uptake was blocked with Lugol s solution

The amount of radioactivity was determined as described in the previous article (9) and expressed as a percentage of the injected dose The amount of urinary protein was estimated by the biuret method. The reported data (Table I) are the mean of the excreted protein on four consecutive days except in patients 21–25 for whom a one-day value is given. There was a considerable variation in urinary protein loss between the patients Patients with a low degree of proteinuria at the time of the present study had a higher excretion at earlier observations but immunologically determined selectivity patterns which may be inaccurate in cases with slight proteinuria, were essentially unchanged Protein clearances in group A were carried out twice during the clinical admission. The method was the same as described earlier from this Department (13 14) and essentially similar to the one introduced by Blainey et al (3) Excretion patterns were defined as selective when the IgG clearance was below 20% of the transferrin clearance and both alpha macroglobulin and alpha 1 lipoprotein were immunologically undetectable in non-concentrated urine samples

Serum creatinine was estimated as mentioned in the previous article (9)

In this study it appeared unnecessary to take into account the quantity of free iodine excreted in urine Both in patients and control subjects the proportion of free iodine averaged 7% of the total excreted radioactivity This was valid for both the LMW PVP and the HMW PVP Free iodine was estimated with an ion exchange resin as well as with dialysis No differences of any importance were found between the ratios LMW PVP/HMW PVP calculated with values corrected for free iodine as compared to non-corrected values

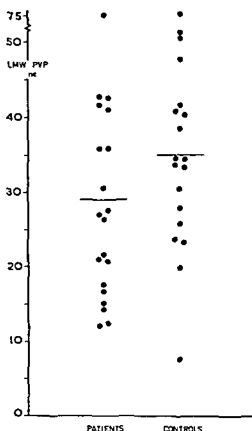


Fig 1 Urinary excretion of LMW PVP during 24 hours after injection in patients of group A and their controls Amounts of PVP are expressed as percentages of the injected dose

RESULTS

Fig 1 summarizes data on the urinary excretion of LMW PVP during the first 24 hours after injection in the patients of group A and their controls In both groups a considerable part of the injected radioactivity is excreted in the urine during this time However in the patient group this quantity is on the average significantly lower than in the control group ($p < 0.05$)

In Fig 2 comparable results for the excretion of HMW PVP are shown Again a substantial although smaller amount was excreted on the first day after injection and likewise this was lower in the patient group ($p < 0.025$)

In seven patients (nos 13–20) and in six control subjects the 24 hour urine collection was divided into three consecutive samples The results are shown in Fig 3 for LMW PVP and in Fig 4

It may be concluded that the permeability in the non selective patient group is different from normal and from the selective group but that there is no difference in permeability between the selective patients and normal subjects

This method therefore may demonstrate differences in permeability between patient groups With the PVP batches available for this study there was some overlap between ratios in selective and non selective proteinurias In individual cases no definite conclusion can be drawn as regards selectivity Probably better results can be expected with very sharp peaks of LMW PVP and HMW PVP without any overlap between the molecular weight ranges of the two types

Because this method can be used to investigate capillary transfer mechanisms in other tissues and organ systems such as the gastro-intestinal tract in which immunological protein clearances are difficult to perform for technical reasons a wider use seems possible

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GLOMERULONEPHRITIS WITH INITIAL LUNG PURPURA (GOODPASTURE S SYNDROME)

Survival of Two Patients out of Four

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Abstract Four cases of glomerulonephritis with initial lung purpura (Goodpasture s syndrome) are presented. Two patients succumbed to their disease after a few months one being treated with corticosteroids and hemodialysis, the other with corticosteroids only The third patient showed complete reversal of both the pulmonary and the renal symptoms 10 months after onset of the disease He received no chemotherapy Now he has been in full time occupation for more than six months The fourth patient, during a period of five years, experienced three episodes of glomerulonephritis with initial pulmonary hemorrhage The symptoms especially the hemoptysis, were far more pronounced during the first episode and seemed to respond favorably to corticosteroid therapy No chemotherapy was given during the last episodes. For the last 3–4 years the patient has been in good health. Patients with severe pulmonary hemorrhage and minor renal symptoms may respond favorably to corticosteroid therapy The grave prognosis in Goodpasture s syndrome is based primarily on autopsy series Benign forms do exist and should not be overlooked or left unpublished.

Glomerulonephritis with initial lung purpura or Goodpasture s syndrome is usually a disease of white men clinically characterized by an insidious course with hemoptysis anemia proteinuria hematuria and increasing dyspnea and azotemia The disease is usually fatal within a few weeks or months the patients dying of renal failure or massive pulmonary hemorrhage (2 5 8) Pathologically the lungs show multiple hemorrhages with hemosiderosis thickening of alveolar septa and numerous hemosiderophores in the alveoli The kidneys give a picture of glomerulonephritis with proliferative changes or hyalinization and fibrosis in more long lasting cases The etiological factors are still unknown but the consistent clinical and pathological findings establish Goodpasture s syndrome as a specific disease entity distinct from other disorders involving the kidneys and lungs such as periarteritis nodosa Wegener s granulomatosis lupus erythematosus disseminatus and idiopathic pulmonary hemosiderosis (2 5 8)

The therapeutic regimens include corticosteroids hemodialysis bilateral nephrectomy with intermittent hemodialysis or renal transplantation However the number of patients reported to have survived the acute illness is only about 25 (1–4 6–11 13–21) the observation period in several cases being only some months

This paper presents four additional patients with glomerulonephritis and initial lung purpura two of whom have survived

CASE REPORTS

Case 1

A 51 year-old quay foreman and previous prize fighter was admitted to hospital on 13 December 1967 He had always been in good health, and annual routine examination findings had been negative He gave a history of common cold coughing, hemoptysis, fever and weakness for two weeks and macroscopic hematuria for three days

He was in good condition but slightly orthopneic The temperature was 39 C and the blood pressure 140/90 mm Hg There were crepitations over both sides of the lower chest. Ophthalmoscopic findings were normal except for a small degenerative spot in the right choroid

Laboratory data were urine specific gravity 1028 proteinuria (0 3) marked hematuria and a few granular casts Hb 13.9 g/100 ml red blood cell count 5 2 mill/mm MCHC 30% white blood cell count 15 600 mm with normal differential count, reticulocytes 1 5% ESR 104 mm/h serum iron 45 μg/100 ml blood urea 32 mg/100 ml, serum creatinine 0.9 mg/100 ml Bleeding

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HAEMODYNAMIC EFFECTS OF PROPRANOLOL AND NITROGLYCERIN IN NORMAL SUBJECTS DURING SUBMAXIMAL EXERCISE

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Abstract The haemodynamic effects of propranolol and nitroglycerin have been studied in four normal subjects during submaximal exercise in the sitting position The rise in heart rate normally seen after nitroglycerin was prevented by propranolol and the reduction in the cardiac output and mean pressure caused by both drugs together was of the same order as that produced by only one of them

Nitroglycerin and related compounds have been the principal drugs in the treatment of angina pectoris for about a century In the last five years however substances with a completely different mode of action β adrenergic blockade have come to play an increasing role in the therapy of angina pectoris (3 4 9 10 11 12 13 16 18 19)

The combination of these two types of drug nitrates and β blocking agents has naturally also been widely used and not only an additive but also a synergistic effect has been reported (1 14 17) The promising results of the combined therapy have been attributed to the fact that both types of drugs reduce the work of the heart nitrates by diminishing peripheral resistance and venous return and β blockers by reducing the force and rate of the heart (17)

In the above mentioned investigations with the combined therapy the haemodynamic studies were however confined to the heart rate (14 17) or the heart rate and the blood pressure (1) It might be expected that the combined use of nitroglycerin and β blockers would cause a precipitous fall in cardiac output In the present study on a few normal subjects some preliminary observations are therefore reported on the haemodynamic effects of propranolol and nitroglycerin when used alone and together during submaximal exercise

METHODS

Four healthy male volunteers, aged 18 25 39 and 51 years were studied The subjects were fasting at the examination, which started in the morning Polythene tubes were inserted in the brachial artery of one arm and in a cubital vein of the other (2) Measurement of the cardiac output was made by a dye-dilution technique using bromsulphthalein as the indicator Systemic arterial pressures were registered by means of inductance manometers and recorded together with the electrocardiogram on a direct writing electrocardiograph (AB Elema Stockholm Sweden) Peripheral resistance was calculated as the quotient of the mean arterial pressure in mm Hg and the cardiac output in l/min

The subjects were studied in the supine position at rest and in the sitting position cycling on an electrically braked ergometer (AB Elema) They were first studied without propranolol at rest and during a graded submaximal exercise working six min on each work load (300 600 or 900 kpm/min one subject only 300 kpm/min) After six min on the highest work load when the cardiac output had been measured they were given nitroglycerin (0 5 mg sublingually (Dumex)) and after another four to six min of continuous exercise on the same load new haemodynamic measurements were made After ten min rest 30 mg propranolol (Inderal) was given per os and the subjects were allowed to rest for another 60 min They were then again examined at rest and during corresponding work loads, again with nitroglycerin on the highest load

RESULTS

The haemodynamic effects of propranolol alone are given in Table I The heart rate was reduced both at rest and during exercise in all four subjects Also the stroke volume was lowered in most cases so that the cardiac output was reduced correspondingly more than the heart rate both at rest and at work except in one subject (case 3

Table I *Haemodynamic data obtained at rest and during exercise in sitt position before and after propranolol*

Case no	Age	Work load (kpm/min)	Cardiac output (l/min) Control	Cardiac output (l/min) Prop	Heart rate (beats min) Control	Heart rate (beats min) Prop	Stroke volume (ml) Control	Stroke volume (ml) Prop	Pressures (mm Hg) Control Systol	Control Diastol	Control Mean	Prop Systol	Prop Diastol	Prop Mean	Peripheral resistance (U) Control	Peripheral resistance (U) Prop
1	51	Rest	6 8	5 9	63	56	108	105	114	69	92	109	65	81	13 5	13 7
		300	10 5	8 8	81	78	130	113	134	75	97	118	68	91	9 2	10 3
2	18	Rest	9 1	6 7	83	68	110	99	176	73	8	111	68	83	9 6	12 4
		300	14 6	9 5	132	102	111	93	153	87	109	131	83	101	7 5	10 6
		600	16 4	9 9	168	124	98	80	149	82	108	128	78	104	6 6	10 5
3	39	Rest	5 1	5 3	70	63	73	84	113	65	84	96	59	72	16 5	13 6
		300	—	—	102	93	—	—	141	78	102	114	66	91	—	—
		600	14 1	11 0	132	110	107	100	157	80	112	128	70	96	8 0	8 7
4	25	Rest	7 9	[illegible]	77	[illegible]	102	99	125	70	92	119	72	91	11 6	12 8
		600	14 3	11 1	117	102	122	109	149	75	100	141	78	101	7 0	9 1
		900	18 4	16 4	140	[illegible]	131	137	151	73	106	153	78	107	5 8	6 5

Table I) who at rest had 5 1 l/min before and 5 3 l/min after propranolol The systolic pressure decreased in practically all cases in the resting as well as the working condition The effect on the diastolic and mean pressures was more varied although in most cases there was a slight de crease As the cardiac output decreased and the mean arterial pressure as a whole was unchanged the calculated peripheral resistance rose in most instances

The circulatory effects of nitroglycerin given during exercise before β blockade are presented in Tables II–VI They are mainly an increase in heart rate and a decrease in cardiac output stroke volume and mean pressure (5 6 15) It has earlier been found (15) that nitroglycerin given twice with 60 min interval is equally effective implying that it is relevant in this study to compare the effect caused by nitroglycerin before and after propranolol was given (Tables II–VI) As also propranolol reduced the cardiac output, it was interesting to see whether these two substances had synergistic effects but when nitroglycerin was given to subjects under β blockade there seemed to be no further decline of the cardiac output What happened was that the in crease in heart frequency normally found after nitroglycerin was prevented There was a slight rise in heart rate from the propranolol value but not at all to the same extent as before β blockade This effect of β blockade has been re ported earlier (1 14 17) When nitroglycerin was given under β blockade the stroke volume did not decrease to the same degree as before pro pranolol but the decline of the mean pressure was of the same order as before the β adrenergic blocking agent was given The increase in periph eral resistance found during propranolol was reduced in two of the four cases by nitroglycerin in one of them it decreased even below the value found before propranolol

COMMENTS

The present observations have confirm d earlier reports on the effects of β blockade during exer cise (7 8) apart from the finding by Epstein et al (8) that there was no consistent alteration of the systemic vascular resistance whi h in the present study was found to increase in most in stances

Table II *Changes in heart rate (beats/min) during exercise after propranolol and nitroglycerin*

Case no	Work load (kpm/min)	Initial value	Propranolol only	Nitroglycerin only	Propranolol + nitroglycerin
1	300	81	−3	+21	+5
2	600	168	−44	+12	−30
3	600	132	−22	+23	−18
4	900	140	−20	+19	−2

Table III *Changes in cardiac output (l/min) during exercise after propranolol and nitroglycerin*

Case no	Work load (kpm/min)	Initial value	Propranolol only	Nitroglycerin only	Propranolol + nitroglycerin
1	300	10.5	−1.7	−2.5	−2.4
2	600	16.4	−6.5	−2.0	−3.6
3	600	14.1	−3.1	−1.3	−2.0
4	900	18.4	−2.0	−2.0	−2.9

Table IV *Changes in stroke volume (ml) during exercise after propranolol and nitroglycerin*

Case no	Work load (kpm/min)	Initial value	Propranolol only	Nitroglycerin only	Propranolol + nitroglycerin
1	300	130	−17	−52	−36
2	600	98	−18	−18	−5
3	600	107	−7	−24	−1
4	900	131	+6	−28	−19

Table V *Changes in mean pressure (mm Hg) during exercise after propranolol and nitroglycerin*

Case no	Work load (kpm/min)	Initial value	Propranolol only	Nitroglycerin only	Propranolol + nitroglycerin
1	300	97	−6	−11	−12
2	600	108	−4	−15	−10
3	600	112	−16	−20	−24
4	900	106	−1	−12	−6

Table VI *Changes in peripheral resistance (U) during exercise after propranolol and nitroglycerin*

Case no	Work load (kpm/min)	Initial value	Propranolol only	Nitroglycerin only	Propranolol + nitroglycerin
1	300	9.2	+1.1	+1.6	+1.3
2	600	6.6	+3.9	−0.1	+1.1
3	600	8.0	+0.7	−0.8	−0.7
4	900	5.8	+0.7	−0.1	+0.7

The synergistic effect on angina pectoris found with the combined therapy β blockade and nitroglycerin or other nitrates is probably due at least in part to the prevention of the oxygenconsuming tachycardia caused by nitroglycerin alone as earlier suggested by MacAlpin et al (14) and Russek (17) It might then be expected that the cardiac output would be very much lowered when using these two drugs together but this suspicion could not be verified in the present investigation instead the decrease of the cardiac output was of the same order as that caused by only one of the drugs This was due to the fact that the reduction of the stroke volume produced by nitroglycerin was less pronounced when also propranolol was given Both drugs are hypotensive but the drop in mean pressure caused by the combination did not seem to be larger than that produced by nitroglycerin alone The rise in peripheral resistance found after propranolol was however not consistently affected when also nitroglycerin was given

The combination of propranolol and nitroglycerin thus seems to produce haemodynamic effects which should be favourable for the ischaemic heart i e decrease in heart rate cardiac output and mean pressure These effects diminish the work of the heart i e the oxygen consumption which can then be better adapted to the available oxygen Other mechanisms may of course also be operative

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PLASMA HYDROCORTISONE VALUES IN HEART DISEASE

Results after Acute Myocardial Infarction (with and without Cardiogenic Shock) and in Patients with Congestive Heart Failure

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Abstract The plasma hydrocortisone level has been examined in ten patients with acute coronary occlusion and shock in 12 patients with acute coronary occlusion without shock, in seven patients with congestive heart failure and in five control patients Owing to the circadian rhythm of the plasma hydrocortisone level the examinations have been carried out in five daily determinations on three consecutive days As compared with the control patients, the average values were found to be significantly higher in patients with congestive heart failure and even higher in the patients with coronary occlusion with and without shock. No correlation was found between the plasma hydrocortisone concentration and the enzymes SGOT and SLDH the morning temperature or the blood pressure in the patients with acute myocardial infarction The plasma hydrocortisone level in the patients with coronary occlusion returned to the normal range within 48 hours after admission The highest values were found in the patients who died and the increased level was maintained until the last determinations before death

In relation to the discussion about the value of treatment with glucocorticoids in patients with shock following acute coronary occlusion it has seemed natural to establish the spontaneous adrenocortical reaction following a myocardial infarction

Several investigators have shown that in most cases of acute myocardial infarction the plasma hydrocortisone level is higher during the first couple of days Logan and Murdoch (7) found that in all their patients the values of plasma hydrocortisone returned to the normal range within three days after the infarction In addition they found a correlation between the peak plasma hydrocortisone values and the corresponding values of SGOT which was also found by Bailey et al (1) Logan and Murdoch determined plasma hydrocortisone shortly after admission and thereafter at frequent intervals Bailey et al took blood samples from most patients at 10 a m for 4–6 days In both these investigations the peak plasma hydrocortisone values were used and no regard was paid to the circadian rhythm of the plasma hydrocortisone level The above mentioned authors state that the patients had acute myocardial infarction but no information is given about the time elapsing from the onset of the symptoms until admission

In our investigation the plasma hydrocortisone values have been determined five times daily and all the patients with coronary occlusion were hospitalized within the first 24 hours after the beginning of the symptoms We have examined a group of patients with acute myocardial infarction with and without shock and we have tried to correlate the plasma hydrocortisone values to the blood pressure and various parameters used in the diagnosis of myocardial infarction Likewise we have examined the plasma hydrocortisone level in patients with congestive heart failure

MATERIAL AND METHODS

The investigation comprises

A Twenty two patients with acute myocardial infarction arisen within the last 24 hours before admission (diagnostic criteria characteristic precordial pains and electrocardiographic changes, increase of the enzymes SGOT and SLDH and increase of temperature)

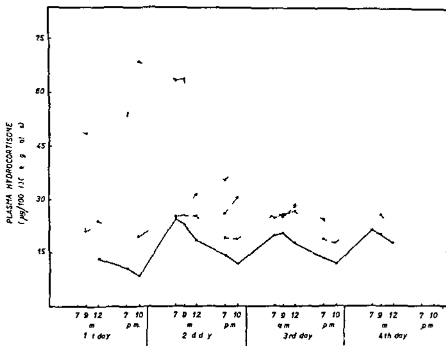


Fig 1 Average values of plasma hydrocortisone in ten patients with acute myocardial infarction and shock (— — — —) twelve patients with acute myocardial infarction without shock ( ) seven patients with congestive heart failure (————) and five control patients (————)

Of these patients

1 ten (5 women 5 men aged 52–72) had cardiogenic shock (systolic blood pressure below 90 mm Hg) at some time during the observation period and twelve (12 men aged 41–72) had no cardiogenic shock

B Seven patients with congestive heart failure without acute myocardial infarction (2 women 5 men aged 39–81)

C Five control patients without pain and without any clinical sign of endocrine disease (2 women 3 men aged 27–53)

Blood samp es for determination of plasma hydrocortisone were taken after admission for three days at 7 a m 9 a m 12 a m 7 p m and 10 p m which meant 15 samples from surviving patients At the same time the blood pressure was measured The patients with shock were treated with intravenous infusion of metaradrin

The blood samples for determination of SGOT and SLDH values in the patients with myocardial infarction were taken in the morning and we tried to correlate these values to the plasma hydrocortisone concentration at 9 a m and to the morning temperature and the blood pressure at 9 a.m

The plasma hydrocortisone estimations were carried

Table I *Mean values of plasma hydrocortisone in five controls (see text)*

	7 a m	9 a m	12 a m.	7 p m.	10 p m
No of observations	14	15	15	15	15
Mean value (μg/100 ml)	22 1	21 3	17 0	12 9	10 9
S D	5 4	4 6	5 0	3 1	4 3

Table II *Mean values of plasma hydrocortisone in seven patients with congestive heart failure*

Calculation of significance between these values and mean values of plasma hydrocortisone in controls

Day after admission	First			Second				
Point of time	12 a m	7 p m	10 p m	7 a m	9 a m.	12 a m	7 p m	10 p m
No of observations	2	7	7	6	7	7	7	6
Mean value (μg/100 ml)	24 0	21 0	19 7	25 0	25 5	25 3	20 9	18 7
S D	0 0	5 6	5 0	4 0	6 6	5 3	9 2	9 3
Significance	$p < 0\ 001$	$p < 0\ 01$	$p < 0\ 05$	n s	n s	$p < 0\ 005$	n s	n s

n s — not significant

out by Medicinsk Laboratorium Copenhagen using the fluorimetric method of De Moor et al. (3 4)

The statistical calculations were done with a computer (IBM 1130)

RESULTS

The average values of plasma hydrocortisone in the different groups of patients are shown in Fig 1

It seems from the curve comprising the controls that the values at the same hour are the same from day to day and by analysis of variance it is confirmed that there is no significant difference We have therefore used the values from all three days when calculating the average values of plasma hydrocortisone in the control patients at *each individual hour and the results appear* from Table I On the other hand the analysis of variance shows a significant difference between the values at the different moments within the same 24 hours ($F = 4\,444$ $p < 0\,01$)

After admission the patients with congestive heart failure have increased plasma hydrocortisone values and even higher values are found in the patients with acute myocardial infarction without shock The highest values are seen in the patients with acute myocardial infarction and cardiogenic shock This appears from Fig 1 and the average values are shown in Tables II III and IV together with the results of the statistical analysis Here the mean plasma hydrocortisone values of the different groups of patients are appraised in relation to the mean values of the control group

The *highest* plasma hydrocortisone value we have recorded was 164 μg per 100 ml The highest values were found in the patients who died and the increase persisted until the last determinations before death In the group with myocardial infarction without shock one patient died (23 days after admission) in the group with myocardial infarction and cardiogenic shock six patients died (from 20 hours to 13 days after admission)

Table V shows the last values of plasma hydrocortisone recorded in the patients who died

Even in the patients with acute myocardial infarction and shock the average values of plasma hydrocortisone return to the normal range within 48 hours Until then the values are significantly higher The range is great in the period when the values are increased but during the first 48 hours all patients with acute myocardial infarction and shock still have values above the mean for controls In the group with acute myocardial infarction without shock we have recorded during the *first 24 hours one plasma hydrocortisone value* within the normal range while during the next 24 hours we found 9 out of 57 observations to be within the normal range

In the patients with acute myocardial infarction with and without shock, we have tried to correlate the plasma hydrocortisone values at 9 a m. to the morning temperature to SGOT to SLDH and to the systolic and diastolic blood pressure The results appear from Tables VI and VII We have found no significant correlation in the patients with coronary occlusion without shock In the patients with acute coronary occlusion and shock we have found a correlation ($p < 0\,05$) between plasma hydrocortisone and the diastolic blood pressure at 9 a m We also found a significant correlation between plasma hydrocortisone and SLDH but after exclusion of a single extremely high SLDH value there was no significance

h rd				
a.m.	9 a m	1? a.m	7 p m	10 p m.
7	6	6	6	6
8 9	25 8	27 0	18 8	17 8
3 1	7 3	2.6	6 2	6 8
s.	n s.	$p < 0\,01$	n s	n s

DISCUSSION

The examinations confirm earlier observations that the plasma hydrocortisone level is elevated in patients with acute myocardial infarction The increase is higher when cardiogenic shock occurs The examination of our control patients shows that admission per se does not influence the plasma hydrocortisone concentration

It is still discussed what causes the increased adrenocortical activity in patients with acute coronary occlusion Logan and Murdoch (7) as well as Bailey et al (1) suppose that the actual myocardial necrosis is the essential factor This

Table III *Mean values of plasma hydrocortisone in twelve patients with acute myocardial infarction without shock*

Calculation of significance between these values and mean values of plasma hydrocortisone in controls

Day after admission	First			Second				
Point of time	12 a m	7 p m	10 p m	7 a m	9 a m	12 a m	7 p m	10 p m
No of observations	6	9	12	12	11	10	12	12
Mean value (μg 100 ml)	39 0	35 0	28 0	34 1	25 6	31 3	26 2	30 7
s.d	9 9	21 5	17 8	18 2	12 7	11 2	1 3	14 1
Significance	$p < 0\ 01$	n s	$p < 0\ 01$	n s	n s	$p < 0\ 05$	$p < 0\ 005$	$p < 0\ 001$

Table IV *Mean values of plasma hydrocortisone in ten patients with acute myocardial infarction and shock*

Calculation of significance between these values and mean values of plasma hydrocortisone in controls

Day after admission	First			Second				
Point of time	12 a m	7 p m	10 p m	7 a m	9 a m	12 a m	7 p m.	10 p m.
No of observations	9	10	9	9	8	8	8	7
Mean value (μg 100 ml)	55 0	53 4	57 4	53 0	58 9	40 4	35 8	39 1
s.d	23 0	29 5	25 4	30 5	28 3	18 3	19 2	18 7
Significance	$p < 0\ 001$	$p < 0\ 01$	$p < 0\ 001$	n s	$p < 0\ 01$	$p < 0\ 01$	n s	$p < 0\ 01$

Table V *The last recorded plasma hydrocortisone values in patients who died*

Diagnosis	Plasma hydrocortisone (μg 100 ml)	Time after admission for Plasma hydrocortisone		Death
Acute myocardial infarction + shock	4[illegible]	7 a m 4th day		7 d
Acute myocardial infarction + shock	130	10 p m 1st day	(9 h before death	0 h
Acute myocardial infarction + shock	74	7 p m. 3rd day	([illegible] h before death)	44 h
Acute myocardial infarction + shock	105	9 a m 2nd day	(1 h before death)	4 h
Acute myocard al infarction + shock	37	7 p m 3rd day		13 d
Acute myocardial infarction + shock	30	9 a m. 4th day		5 d
Acute myocardial infarction without shock	38	10 p m 3rd day		3 d
Congestive heart failure	4	7 a m 3rd day		7 d

Table VI *Twelve patients with acute myocardial infarction without shock*

Plasma hydrocortisone correlated to blood pressure rectal temperature SGOT and SLDH

Variable *Y*	Variable *X*	No of observations (*n*)	Correlation coefficient (*r*)	Probability (*p*)
Plasma hydrocortisone at 9 a.m.	Systolic BP at 9 a.m	35	−0 0169	n s
Plasma hydrocortisone at 9 a.m.	Diastolic BP at 9 a.m	35	−0 1802	n s
Plasma hydrocortisone at 9 a.m.	Morning temperature	32	−0 0505	n s
Plasma hydrocortisone at 9 a m.	SGOT	35	0 1433	n s
Plasma hydrocortisone at 9 a.m	SLDH	34	0 0 56	n s

Third				
7 a.m	9 a.m.	12 a.m	7 p m	10 p m
11	9	12	12	10
50	25 9	28 7	25 8	22 1
91	10 1	9 0	10 7	11 2
ns	n s	$p < 0\ 001$	$p < 0\ 01$	n s

Third				
a m	9 a m	12 a m	7 p m	10 p m
7	7	7	7	6
97	25 3	28 9	24 4	20 0
71	9 5	9 6	9 1	9 2
s	n s	n s	n s	n s

is supported by the fact that these investigators did not find any increased level of plasma hydro cortisone in patients with precordial pain without myocardial infarction In the same materials there was a correlation between the peak plasma hydro cortisone values and the peak SGOT values

Oka (8) however who determined the free 17 hydroxycorticosteroids in plasma also found in creased values in patients with stenocardia with out any sign of myocardial infarction

We have not been able to demonstrate a correlation betwe n the plasma hydrocortisone level and the SGOT concentration We have chosen to compare values taken at the same time instead of using the peak values This is also the case with regard to SLDH and furthermore as the exami nation period was three days we have in several cases most likely not recorded the peak SLDH value at all as it might appear later The correla tion we have found b tween the plasma hydro cor isone level and the diastolic blood pressure in patients with acute myocardial infarction and sho k is hardly real All the patients with shock were treated with metaradrin at different times during the observation period The blood pressure figures are therefore influenced by the treatment and do not indicate the spontaneous course

We have thus be n unable to show any signifi cant correlation between the plasma hydro orti sone level and the other parameters used in our examination of patients with acute coronary oc clusion

We have found the highest plasma hydrocorti sone values in the patients who di d This might support the presumption made by Klein and Pal mer (5) that the prognosis is worse if the initial plasma hydrocortisone concentration exceeds 40 μg per 100 ml However several of our patients survived with higher values

In the material of Bailey et al (1) there was one patient who died 11 hours after admission with a normal level of plasma hydrocortisone im mediately before death In our investigation all the patients who died had increased values at the last determinations

If the myocardial necrosis causes the increase of plasma hydrocortisone the question arises why

Table VII *Ten patients with acute myocardial infarction and shock*

Plasma hydrocortisone correlated to blood pressure rectal temperature SGOT and SLDH

Variable Y	Variable X	No of observations ( )	Correlation coefficient ( )	Probab lity (p)
Plasma hydrocortisone at 9 a m	Systol c BP at 9 a m	24	0 3944	n s
Plasma hydrocortisone at 9 a m.	Diastolic BP at 9 a m	0	0 52_6	$p<0\ 05$
Plasma hydrocort sone at 9 a m.	Morn ng temperature	23	0 3197	n s.
Plasma hydrocortisone at 9 a m	SGOT	22	0 4162	n s
Plasma hydrocortisone at 9 a m	SLDH	22	0 5938	$p<0\ 01$
Plasma hydrocortisone at 9 a m. after deduction of 1 pat ent with extremely h gh SLDH value	SLDH	21	0 1348	n s

the patients with congestive heart failure also have increased values Among our patients in this group the average values of plasma hydrocortisone are significantly higher during the first 24 hours after admission These patients had most likely no myocardial necrosis

Knapp et al (6) examined the circadian rhythm of plasma 11 hydroxycorticosteroids in 10 patients with congestive heart failure The average values were determined on the basis of seven observations in 24 hours The curve did not show the characteristic variation The values were within the normal range from 8 a m to 4 p m After that time the level was above normal Contrary to the above mentioned examination several values in our patients were increased also about noon

Connolly and Wills (2) examined plasma cortisol at midnight and at 9 a m after the first day in hospital in six patients with congestive heart failure (one patient with myocardial infarction) All the patients had increased values at midnight and three (including the patient with myocardial infarction) had also values above normal at 9 a m The authors draw attention to the fact that with the technique used by them both cortisol and corticosterone were determined This is also the case in our investigation Congestive heart failure can be followed by secondary hyperaldosteronism and therefore the estimated high level may be due to an increased corticosterone concentration

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Acta med scand Vol 186 pp 417–420 1969

THE VALUE OF PBI AND T_3 TEST IN AN ENDEMIC GOITER AREA

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Abstract An investigation of the value of the PBI and of the tri-iodothyronine (T_3) test in an endemic goiter area shows that in euthyroid and hypothyroid cases PBI gives pathologically raised values in 30% The T test in the same material gave incorrect values the figure usually being too low in 8% of the euthyroid cases In the whole material (340 patients) PBI gave incorrect information in 28% the T test in 9% and free thyroxin index" (PBI x T_3) in 7% In an endemic goiter area the diagnosis of the functional condition of the thyroid is considerably more difficult since tracer iodine examination as well as PBI determination often give values expressing hyperthyroidism in euthyroid cases. Clinical assessment of the patients is therefore of specially great value in an endemic goiter area

In an endemic goiter area our usual laboratory methods in thyroid diseases not seldom give incorrect results In iodine tracer examinations one fairly often gets hyperthyroid values i e the I^{131} taken up by the thyroid gland is increased and that which is excreted in the urine is reduced in spite of the patient being euthyroid In a work published earlier we have shown that this occurred in 51 out of 256 patients (7) PBI^{131} determination in these cases has proved to be a valuable complement (10) but here too one can get raised values in euthyroid patients as in cases of small active adenomata or after treatment with radioactive iodine

Raised PBI values are not a rare occurrence in euthyroid patients with endemic goiter (6 13) The cause of the raised PBI values would seem to be that the protracted lack of iodine has damaged the thyroid gland so that biologically inactive or low active iodine compounds are released into the blood and are included in the determination Mono- and di iodotyrosine together with thyroglobulin do not normally occur in the blood but may appear in the event of injury to or disease of the thyroid

If mono-iodotyrosine is added in vitro one may find in PBI about 20 % of the added activity while of added di iodotyrosine 50% may be found in PBI Consequently the PBI value is influenced considerably by the low active di iodo- and mono-iodotyrosine pole (8)

The determination of BEI is in this respect much more accurate since mono- and di iodotyrosine practically speaking are not included in this method at all Determination of the BEI value is however a far too complicated method for routine use

Dowling et al found a raised PBI value in five euthyroid adults with goiter and some of them exhibited a nonbutanol-extracted iodised peptide or protein of thyroid origin These patients also had a high uptake of I^{131} (5)

The adsorption of radio-active tri iodotyronine to Sephadex after being added to serum (T_3 test) is used generally as a thyroid function test and lacks a number of the disadvantages of PBI e g sensitivity to iodine contamination The T_3 test reflects the degree of unsaturated thyroxin binding protein (TBP) This test also has its limitations due chiefly to changes in TBP which are not caused by the thyroid When TBP increases so also does PBI to a certain degree while the T_3 value is reduced (e g pregnancy contraceptive pills) (4 9) On reduction of TBP (e g nephrosis) PBI is reduced while the T_3 value is raised (14)

Clark and Horn (3) suggested a so-called free thyroxin index calculated as the product of the values for PBI and T_3 This is an arbitrary figure which should be proportional to free thyroxin in the blood

Nosslin (12) found correct values for PBI in 84 for the T_3 test in 85 % and for free thyroxin index in 91 The material which is not from an endemic goiter area comprised 23 hypothyroid

h product of PBI × T_3 in the various

(..lue 4–10)

	Total no	Too high	Too low
d	284	70	1
thyroid	73	-	—
perthyroid	33	–	1

Table IV *The whole material*

	Right	Wrong	Error ( )
PBI	245	95	28
T_3	308	32	9
PBI × T_3	316	24	7

DISCUSSION

In an endemic goiter area PBI is a bad standard of value regarding the patient s thyroid status especially in differentiating between hyper or euthyroidism In our material PBI was raised in 30% of euthyroid patients without any iodine contamination or treatment being traceable which could effect the determination In seven cases of 23 (30 %) of hypothyroidism PBI was above the lower normal limit

The T_3 test seems to us to be a valuable com plement as in the whole material this test was only wrong in 9% Among the euthyroid patients (284 in total) there were five above and 19 below the normal limits which implies an error of 8% Also with hypothyroidism the T_3 test seems to be more reliable as in our material only 9 % of the T_3 values were raised while the corresponding figure for PBI here was 30%

The product of PBI and T_3 (free thyroxin index) in this investigation does not give much better values than the T_3 test This is due to two reasons The first is the high percentage of raised PBI values in our material whereby the product also becomes raised The second is that as far as possible we excluded from the material those per sons concerning whom there was reason to suspect disturbances in TBP In these cases on the con trary the product of the PBI and T_3 test gave considerably better results than the PBI and T_3 values alone since disturbances in TBP change the values of PBI and T_3 in the opposite direction

Our investigations of the reliability of [illegible] laboratory methods in an endemic goiter [illegible] have shown that PBI and tracer iodine [illegible] tions give incorrect values in the hyperthyr [illegible] rection The determination of PBI[131] a d the iodothyronine test give considerably less rate values In spite of all new laborator tions which have come into being for [illegible] function of the thyroid the clinical asse has its great value and is a necessary [illegible] portant factor in the determination of th [illegible] function

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Table I *The PBI values for the various groups*

PBI (normal value 4 8)

	Total no	Too high	Too low
Euthyroid	284	86 (30)	0
Hypothyroid	23	7 (30)	—
Hyperthyroid	33	—	2

77 euthyroid and 41 hyperthyroid cases He considers that the index seems to be of special value in cases where both PBI and T_3 test are disturbed because of an abnormal TBP concentration such as is found in pregnancy women taking contraceptive pills nephrosis cirrhosis etc

Also Andersson (1) considers that the product of PBI × T_3 forms a very useful indirect determination of free thyroxin and thereby of the patient s thyroid status This idea is also shared by Wellby and O Halloran (16)

As we have not infrequently obtained PBI values deviating from the clinical picture we have wished to correlate these to the value of the T_3 test and free thyroxin index (the product of PBI × T_3)

MATERIAL

All patients upon whom PBI and T tests had been mad during the period February 1965 to June 1966 inclusive have been clinically judged by one or more doctors in the Medical Clinic Sundsvall Hospital, which is situated in an endemic goiter area All patients with suspected iodine contamination and those who had been treated with oestrogens (e g. contraceptive pills) androgens and thyroid substitute have been excluded from the material Those with seriously changed serum proteins and thereby presumed TBP displacements as well as patients with cirrhosis of the liver nephrosis and malignity have not been included Pregnant patients have likewise been excluded No operated or I^{131} treated case is included except in the hypothyroid material, since otherwise this latter group would have been very small Only patients for whom a definite clinical diagnosis could be made with regard to the thyroid function have been included.

After this thinning-out, the material consisted of 340 patients of whom 284 had been diagnosed as euthyroid, 23 as hypothyroid and 33 as hyperthyroid The clinical diagnosis was confirmed in the two latter groups by cholesterol in the serum iodine tracer examinations, ECG and the result of specific treatment. In cases where we found very high PBI values and iodine supplies could not be anamnestically excluded iodine-tracer examinations have been carried out. Patients with signs of blocking have been assumed to be iodine-contaminated and excluded from the material

METHODS

PBI has been determined by a method Skans and Hedenskog (15) which is a mod a method worked out by Barker (?) The for healthy individuals is 5 9 μg per

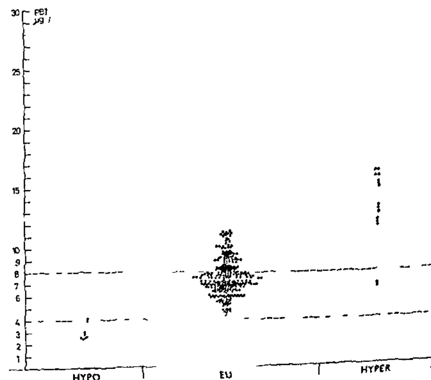


Fig 1

standard deviation of 0 7 μg per 100 ml for both sexes The normal limits are 3 8–8 μg per 100 ml In practice the limits have been set at 4–8 μg per 100 ml

Tri iodothyronine determination (T test) was carried out by a method described by Nosslin (11) in which Sephadex was used to adsorb tri iodothyronine which is not bound to serum protein. The result is expressed in percent and the normal value is 80–120% Lower values are seen in cases of hypothyroidism or raised TBP and higher values in cases of hyperthyroidism or lowered TBP

Free thyroxin index according to Clark and Horn (3) has been calculated as the mathematical product of PBI and T divided by 100

RESULTS

The PBI values for the various groups are shown in Table I and Fig 1

In both the euthyroid and the hypothyroid groups not less than 30 had too high values while in no case was PBI incorrectly low In 6% of the hyperthyroid cases too low PBI values were found

Table II and Fig 2 show the values for the T_3 test

The T_3 test gave incorrect values in 8% of the euthyroid cases and in the majority of these cases the value was too low In the hypothyroid cases

Table II *The T_3 test values for the various groups*

T_3 test (normal value 80–120)

	Total no	Too high	Too low
Euthyroid	284	5	19
Hypothyroid	23	2	—
Hyperthyroid	33	—	6

the value was too high in 9% and in the hyper thyroid patients too low in 18%

Table III shows the product of PBI × T_3 in the various groups

The calculated percentage error for free thy roxin consequently means that 7% of the euthy roid patients had a value which was wrong being almost exclusively too high Furthermore a too high product value was found in 9% of the hypo thyroid cases and too low a value in 3% of the thyrotoxic patients

Table IV shows the number of inaccurate values in the whole material partly in actual figures and partly in percentages

Consequently PBI gave an inaccurate value in 28 of the material the T_3 test in 9% and the free thyroxin index in 7%

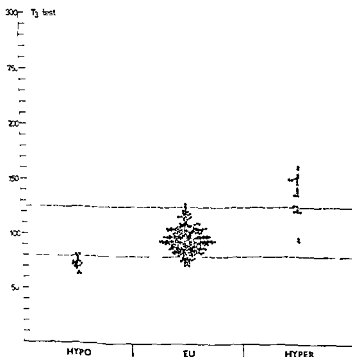


Fig T test values for the various groups.

Table III *The product of PBI × T_3 in the various groups*

PBI × T_3 (normal value 4 10)

	Total no	Too high	Too low
Euthyroid	284	20	1
Hypothyroid	23	2	—
Hyperthyroid	33	—	1

Table IV *The whole material*

	Right	Wrong	Error (%)
PBI	245	95	28
T_3	308	32	9
PBI × T_3	316	24	7

DISCUSSION

In an endemic goiter area PBI is a bad standard of value regarding the patient's thyroid status especially in differentiating between hyper or euthyroidism In our material PBI was raised in 30% of euthyroid patients without any iodine contamination or treatment being traceable which could effect the determination In seven cases of (30%) of hypothyroidism PBI was above the lower normal limit.

The T_3 test seems to us to be a valuable complement, as in the whole material this test was only wrong in 9% Among the euthyroid patients (284 in total) there were five above and 19 below the normal limits which implies an error of 8% Also with hypothyroidism the T_3 test seems to be more reliable as in our material only 9% of the T_3 values were raised while the corresponding figure for PBI here was 30%

The product of PBI and T_3 ("free thyroxin index") in this investigation does not give much better values than the T test This is due to two reasons The first is the high percentage of raised PBI values in our material whereby the product also becomes raised. The second is that, as far as possible, we excluded from the material those persons concerning whom there was reason to suspect disturbances in TBP In these cases on the contrary the product of the PBI and T_3 test gave considerably better results than the PBI and T_3 values alone since disturbances in TBP change the values of PBI and T_3 in the opposite direction.

Our investigations of the reliability of different laboratory methods in an endemic goiter area have shown that PBI and tracer iodine examinations give incorrect values in the hyperthyroid direction The determination of PBI[131] and the triiodothyronine test give considerably less inaccurate values In spite of all new laboratory investigations which have come into being for judging the function of the thyroid the clinical assessment still has its great value and is a necessary and important factor in the determination of the thyroid function

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UREMIC PERICARDITIS

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Abstract The frequency of uremic pericarditis and complicating cardiac tamponade has been determined in a series of 125 patients in terminal uremia. Forty (32) were found to have uremic pericarditis and five of these patients (12.5) developed a pericardial effusion sufficient to produce cardiac tamponade. The diagnosis uremic pericarditis had been made during life in 32 of the 40 patients on the presence of a pericardial friction rub, which was found to be the most constant symptom of pericarditis. Demonstration of acute pericardial constriction was solely clinical: rapidly developing cardiac insufficiency in a clinical situation where hypertensive heart disease, overhydration and severe anemia could be ruled out as etiologic factors.

Seven of the patients with uremic pericarditis were treated with regular hemodialysis using universal heparinization. One patient developed cardiac tamponade in connection with his first hemodialysis. All the rest of the hemodialyses in this patient and in the other patients were without complications. The risk of universal heparinization must therefore be considered to be minimal. The size and shape of the heart became normal in three of the seven patients and the pericardial friction rub disappeared in all of them during intermittent hemodialysis. It is thus possible with effective hemodialysis to treat uremic pericarditis and complicating pericardial effusion so that renal allotransplantation can be carried out. Eight patients with uremic pericarditis in this series, including two with cardiac tamponade, have received renal transplants. Seven of these patients are alive one–three years post transplant.

Pericarditis commonly accompanies terminal uremia. The presence of this complication was previously considered to mean that death was impending (12). Intermittent dialysis and renal transplantation have, however, changed the prognosis of terminal uremia and of the complications that accompany it, so that uremic pericarditis can now be considered accessible to treatment (1, 2, 3, 4, 5, 6, 7, 11).

In the present work a series of uremic patients is analysed in order to determine the frequency and clinical manifestations of uremic pericarditis as well as to elucidate the treatment of this condition.

MATERIAL AND METHODS

In the period April 1964 to October 1968, 125 patients with terminal uremia, i.e. $Cl_C < 5$ ml/min, were admitted to this department. There were 62 men and 63 women with ages ranging from 16 to 87 years. The etiology of the renal diseases involved is given in Table I. Seventy-five of these patients were treated with intermittent peritoneal dialysis, 36 with intermittent hemodialysis and in 65 renal allotransplantation was carried out.

Pericarditis was diagnosed in 40 of these 125 uremic patients (32%). 33 of the 40 died and autopsy was performed on 32. General data concerning these 40 patients is given in Table II. In 32 patients the diagnosis of uremic pericarditis was made in vivo on the presence of a pericardial friction rub; in the other eight the diagnosis was based on autopsy findings. There was no apparent difference between the 40 uremic patients who developed pericarditis and the 85 who did not: both groups were of approximately the same age and sex distribution, the renal diseases involved were not different and the severity of uremia was the same in both groups.

Five of the patients with uremic pericarditis developed an acute pericardial constriction syndrome (12.5).

RESULTS

Pericarditis

A pericardial friction rub was heard in 32 out of 40 patients (80%). The friction rub varied in strength from barely audible to loud enough to be heard with the stethoscope above the chest wall and to be accompanied by a palpable thrill. Pain was not a pronounced symptom, as only 14 of the 40 patients complained of it. When felt, pain was localized to the precordium and was often described as oppressive. Only one patient complained of intense precordial pain with radiation to the back and left shoulder. In almost all the patients pain increased on deep breathing.

Table I *125 patients in terminal uremia etiology*

Chronic glomerulonephritis	45
Acute glomerulonephritis	3
Chroni pyelonephritis	51
Medullary cystic disease	5
Renal hypoplasia	5
Hereditary nephritis	4
Nephrosclerosis	3
Obstructive nephropathy	2
Renal amyloidosis	2
Diabetic nephropathy	2
Polycystic kidneys	1
Renal sarcoidosis	1
Radiation nephritis	1

Table II *Uremic pericarditis general data*

Total patients	125
No with pericarditis	40
Males	19
Females	21
Age range (y)	16–68
Incidence ( )	32
Renal disease	
Chronic glomerulonephritis	19
Chronic pyelonephritis	14
Medullary cystic disease	2
Polycystic kidneys	1
Renal hypoplasia	1
Diabetic nephropathy	1
Renal amyloidosis	1
Renal sarcoidosis	1

Electrocardiograms were taken frequently in all patients and abnormalities were seen in 18 (45%) (Table III) Characteristic signs of peri carditis in the form of S–T segment elevation were seen in ten patients These changes were fol lowed in the course of three to 14 days by de depression and inversion of T waves in six pa tients Low voltage appeared in four patients three of which proved to have a pericardial ef fusion A normal electrocardiogram—excepting the changes secondary to arterial hypertension—was seen in 22 patients In six patients disturb-ances in cardiac rhythm auricular flutter auric ular fibrillation or nodal rhythm were seen Two patients had in addition right axis deviation and one had a right bundle branch block

Thirty-eight patients (95%) evidenced cardiac enlargement of varying degree (Table III) In seven of these patients X ray films suggested a pericardial effusion i e there was a flattening out of the cardiac contour The characteristic tent shaped cardiac silhouette was seen in only three cases

Earlier the most important consequence of the diagnosis of uremic pericarditis was that it in dicated the fatal outcome of uremia in the course of a few weeks Survival before the advent of dialysis was among our patients two to 30 days In three patients however we have seen transitory pericarditis in association with dehydration acidosis infection and papillary necrosis After correction or disappearance of these conditions the symptoms of pericarditis were no longer present One of these patients died a year later because of uremia The other two did not develop uremic pericarditis during the following 2½ years *in spite of progressive renal failure*

Cardiac tamponade

This was seen in five patients Azotemia was most marked among this group creatinine clear ance was < 2 ml/min the mean value for plasma creatinine was 21 0 mg/100 ml and for blood urea 410 mg/100 ml (Table IV) Pericarditis had been present in all of these patients for some length of time how long cannot be stated as many patients had pericarditis on admission

Table III *Clinical features of uremic pericarditis*

Presenting feature	
Friction rub only	11
Friction rub and chest pain	14
ECG abnormalities total no	18
S–T elevation	10
Inversion of T waves	6
Low voltage	4
Arrhythmia atrial flutter	5
nodal rhythm	1
Right axis	2
Normal electrocardiogram	22
X ray	
Cardio-thoracic ratio ≤0 50	2
Cardio-thoracic ratio >0 50–<0 60	22
Cardio-thoracic ratio >0 60–<0 70	13
Cardio-thoracic ratio >0 70	3
Suggestion of pericardial effusion	7
Pain	
Absent	27
Present	13
With radiation	1
Cardiac tamponade	5
No of pericardiocenteses	6
Death resulting from tamponade	3

Left ventricular enlargement from long standing hyperten sion was disregarded

Table IV *Comparison of mean laboratory values at onset of pericarditis with those at time of clinical disappearance in patients in terminal uremia*

	Onset		Disappearance	
	Plasma-creatinine (mg/100 ml)	Blood-urea (mg/100 ml)	Plasma-creatinine (mg/100 ml)	Blood-urea (mg/100 ml)
Group I (17 patients) Pericarditis persisting until death	14 0	325		
Group II (23 patients) Pericarditis treated with dialysis	20 5	340	10 5	152
Group III (5 patients) Pericarditis associated with cardiac tamponade	21 0	410	15 0	250

There was roentgenographic evidence of increasing cardiac size during the days immediately preceding the development of the acute constriction syndrome In two cases the electrocardiogram showed low voltage and in two other patients there was S–T segment elevation and inverted T waves Two patients had auricular fibrillation The clinical symptoms of cardiac tamponade developed in these patients during the course of a few hours and were characterized by signs of right heart failure with dyspnea marked engorgement of neck veins and an enlarged liver The patients were lethargic gray sallow in color and had a falling blood pressure and pulse pressure and increasing peripheral venous pressure The friction rub decreased or disappeared in connection with the appearance of the pericardial effusion

One patient developed an acute constriction syndrome during his first hemodialysis with universal heparinization (Table V case 1) A few days after pericardiocentesis intermittent hemodialysis with universal heparinization was again started Sixteen complication free dialyses were performed azotemia was brought under control and the symptoms of uremic pericarditis disappeared In another patient (Table V case 6 and Fig 1) cardiac tamponade appeared during insufficient intermittent peritoneal dialysis On intensive hemodialysis treatment the uremic pericarditis disappeared and the heart size became normal

The acute constriction syndrome was recognized in vivo in four patients but in one patient the diagnosis was first made on autopsy Pericardiocentesis was done six times with the removal of from 225 to 1360 ml of serosanguinous fluid This procedure had prompt clinical and circulatory effect No complications were observed in connection with pericardiocentesis

Recovery from pericardial effusion and tamponade

Two characteristic cases of uremic pericarditis with accompanying cardiac tamponade are reviewed below

Case 1

A 29 year-old man was discovered to have renal disease in 1960 at the age of 21 The patient was clinically well until July 1966 when advanced renal insufficiency was demonstrated On admission to our department on 5 August 1966 the plasma creatinine was 17 5 mg/100 ml and there was severe hypertension with grade IV hypertensive retinopathy and retinal detachment together with overhydration

The azotemia was controlled by intermittent peritoneal dialysis and the blood pressure reduced to 150–160/105–115 mm Hg on treatment with antipressor agents There was no clinical or roentgenologic evidence of pericarditis X ray of the chest 21 days after admission revealed a slightly increased cardiac diameter (cardio-thoracic ratio 17 3/33 0 cm)

Forty days after admission the blood pressure began to fall and in the course of the following six days dropped from 155 110 to 1 0/90 and the pulse increased from 80 to 120 per min A chest X ray 45 days after admission showed a tent-shaped cardiac silhouette and there was S–T segment elevation but normal voltage

Table V *Patients with pericarditis and roentgenological evidence of pericardial effusion treated with hemodialysis and universal heparinization*

At start of hemodialysis			Pretransplantation			
Case no	Average creatinine clearance (ml min)	Chest film suggestive of pericardial effusion	No of dialyses	Cardiac tamponade during hemodialysis	Chest film showing cardiac dilatation	Outcome
1	1 –	+	17	+	+	Transpl 18 11 1966 Normal renal function Creatinine clearance 70 ml min
2	0–1	+	14	–	+	Transpl 7 11 66 Normal renal function Creatinine clearance 65 ml/min
3	0–1	–	7	–	–	Transpl 24 1 68 Normal renal function Creatinine clearance 100 ml min
4	1–2	–	2	–	+	Transpl 28 2 68 Normal renal function Creatinine clearance 60 ml min
5	3–4	–	51	–	+	Second transpl 20 10 68 Creatinine clearance 30 ml min
6	O	+	27	–	–	Transpl 28 8 68 Normal renal function Creatinine clearance 70 ml/min
7	1–2	–	14	–	–	Transpl 8 3 67 Initially normal renal function Since August 1967 chronic rejection Died 1 2 68

n the electrocardiogram. The plasma creatinine was 17 5 mg 100 ml at this time

The following day the patient felt short of breath. At the start of hemodialysis no 1 with universal heparinization, the patient collapsed the blood pressure was 70 40 and the pulse weak—approx. 90/min. The patient complained of pain in the left side of the chest He was listless and his face had a pale gray color Respiration was weak and irregular The heart sounds were indistinct the apical beat was felt at the anterior axillary line *No pericardial friction rub was heard* The clinical condition remained unchanged during the following hour and therefore pericardiocentesis was performed with the removal of 1360 ml of sero-sanguinous fluid. There was a prompt clinical and circulatory effect. the blood pressure rose to 140 80 and the pulse became strong and regular—approx. 100 per min. Roentgenographically no change in the cardiac form or size could be demonstrated after pericardiocentesis.

The patient's general condition improved during the course of the following months as a result of intermittent hemodialysis. Renal allotransplantation was performed 103 days after admission and now two years later the patient has normal graft function (Cl_c 70 ml min) and the blood pressure is normal The retinal lesions have healed and the heart has a normal form and size on X ray The patient has returned to work and is fully employed

Comment

After precursory roentgenological and electrocardiographical evidence of increasing pericardial effusion this patient developed an acute pericardial constriction syndrome during his first hemodialysis with universal heparinization After pericardiocentesis 16 additional hemodialyses with universal heparinization were performed without complications The heart decreased in size during dialysis treatment but normal cardiac form and size was first obtained after renal allotransplantation

Case 6

A 30-year-old man, who at the age of 5 developed acute glomerulonephritis after an attack of scarlatina

On 6 May 1968 the patient was admitted in terminal uremia with a plasma creatinine of 30 5 mg 100 ml He was severely overhydrated Blood pressure was 200 120 mm Hg and the eyegrounds revealed grade IV hypertensive changes The patient was oliguric on admission and, from the 5th post admission day anuric Intermittent dialysis was started immediately and after the first three peritoneal dialyses the patient had sustained a weight loss of ?? kg, but marked azotemia was still present (Fig. 1)

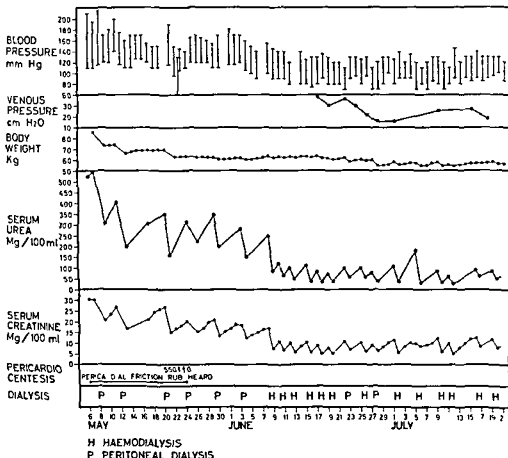


Fig 1 Case 6 The clinical course in a 30 year-old man who on admission on 6 May 1968 was severely over hydrated and markedly uremic Pericardiocentesis was performed on May 2_ and 23 because of cardiac tamponade which developed as a complication of uremic pericarditis Durin_ intensive chronic dialysis azotemia was controlled and the signs of pericardial constriction lessened Bilateral nephrectomy was carried out on 5 July 1968 and renal allotransplantation 23 days later Four weeks after transplantation the creatinine clearance was 60 ml/min the blood pressure normal and the size and shape of the heart were within normal limits

A pericardial friction rub was present on admission and persisted despite dialysis Increasing neck vein engorgement and progressive cardiac enlargement were noted. On the evening of the 15th day in hospital the patient complained of oppressive chest pain and dyspnea His condition worsened rapidly in the course of the next few hours Blood pressure fell from 160/100 to 125/60 *the apical beat could not be felt*, the pericardial friction rub decreased in strength the neck veins were engorged and the liver margin was felt 5–6 cm below the right costal marg n The patient was ashen in color and severely dyspneic On pericardiocentesis 550 ml sero-sanginous fluid was removed His condition improved somewhat thereafter The following day an X ray film of the chest showed increasing cardiac enlargement and again there were clinical signs of right heart failure Pericardiocentesis was aga n perform d, but this time it was not possible to aspirate flu d.

The cl n cal condition remained critical but on intensification of dialysis it was possible to eliminate the overhydration and to keep the azotemia at a sufficiently low level (Fig 1) The venous pressure fell liver size decreased and the cardiac silhouette became normal (Fig 2 *b*) The patient was treated with intermittent hemodialysis for 3 / months Universal heparinization was used during all dialyses without complication On 5 *August 1968* bilateral nephrectomy was performed and 23 days later the patient received a d)nor kidney from his mother Graft function is now two months later good with a creatinine clearance of 60 ml/min. Blood pressure is normal and X ray of the chest shows a heart of normal size and shape

Comment

This patient was s verely uremic and overhydrated on admission



Fig 7 (a) Case 6 X ray of the chest taken in the supine position on 2_ May 1968 before the removal of 550 ml sero-sanguinous fluid from the pericardium. There is marked cardiac enlargement An invagination is still seen between the aortic arch and the right atrium and thus the characteristic tent-shaped cardiac silhouette is not present (b) Case 6 X ray of the chest taken in the standing position on 18 July 1968 The heart measures 15 4 cm the chest 3? 5 cm in diameter

In spite of two weeks of intermittent dialysis his condition remained essentially unchanged until the 15th day after admission when he developed acute pericardial constriction which necessitated pericardiocentesis Azotemia was later brought under control during regular hemodialysis After successful renal allotransplantation the blood pres sure and cardiac size became normal

Autopsy findings

Eighty five of the 125 patients in the series died autopsy was performed in 84 and evidence of pericarditis was found in 32 (38) In _4 patients the diagnosis had been made during life Fibrinous or sero-fibrinous changes were found in 21 Fibrinous or healed peri carditis with numerous synechiae was seen in 11 and in eight these changes were so marked that the pericardial cavity was completely obliterated. Calcification of the pericardium was not seen In eight patients (25%) there was autopsy evidence of pericarditis but no pericardial rub had been heard and the diagnosis was therefore made post mortem Severe fibrinous pericarditis with total obliteration of the pericardial cavity was found in seven patients and pericardial fluid in one

Table VI *Character of pericardial effusion*

Autopsy and pericardiocentesis (total 10 pats.)		
Serous	3	(range 60-100 ml)
Sero-hemorrhagic	5	(range 125-1500 ml)
Hemopericardium	0	
Purulent	2	(range 400-700 ml)

Pericardial fluid

This was obtained from ten patients either by peri cardiocentesis during life or at autopsy The fluid was sero-sanguinous in most cases and the volume varied from 60 to 1500 ml Cardiac constriction was seen in one case where the pericardial cavity contained 1500 ml of sero-sanguinous fluid Two patients demonstrated purulent pericarditis as a complication of a Staphylococcus septicemia True hemopericardium was not seen (Table VI)

DISCUSSION

Pericarditis was demonstrated in 40 of 125 patients with terminal uremia (32 %) This frequency of occurrence is in accordance with the findings of others (2 3 12) In five out of the total 40 cases of uremic pericarditis life threatening acute pericardial constriction due to cardiac tamponade developed

The reason why pericarditis develops in some uremic patients and not in others and likewise why effusion develops in some cases of peri carditis and not in others is unknown In our series pericarditis was seen in patients who were severely uremic and an acute pericardial con

striction syndrome developed among the most severely uremic of these patients ($Cl_{Cr}<2$ ml/min)

The diagnosis of uremic pericarditis is made by auscultation as the most constant symptom is the presence of a pericardial friction rub Generally there are no subjective symptoms but some patients do complain of precordial oppres sion Clear cut electrocardiagraphic and roent genologic changes appear only when pericarditis is complicated by effusion

The speed with which fluid accumulates and thus the amount of pressure produced in the pericardial cavity determines the severity of symptoms in cases of pericardial effusion The volume of the effusion seems to be of less im portance Similarly the hemodynamic changes which determine the clinical symptoms are the result of pressure changes Diastolic filling of the ventricles is diminished because of increased intra pericardial pressure At the same time the diastolic pressure in the right ventricle increases and this produces a pressure increase in the right atrium and thereby an increase in the peripheral and central venous pressure Cardiac output is temporarily maintained by an increase in fre quency and by peripheral vasoconstriction but when a critical intra pericardial pressure is reached cardiac output decreases the blood pres sure falls and circulation stops Pericardiocentesis with the aspiration of the effusion permits rapid and complete reestablishment of circulation by reducing the pressure in the pericardial cavity

Slow development of a pericardial effusion ac companied by only a slight rise in intra pericardial pressure may be difficult to differentiate from the cardiac enlargement resulting from arterial hypertension severe anemia and overhydration—all of which are common in terminal uremia In two of our patients a slow accumulation of fluid in the pericardium was manifested by disappear ance of the normal cardiac contours on X ray without there being any symptoms or physical signs of cardiac constriction (Table IV cases 2 and 7)

The demonstration of acute cardiac constric tion in our patients was made solely on clinical observation the rapid development of cardiac in sufficiency in the course of a few hours with dyspnea precordial oppression engorged neck veins hepatomegaly falling blood pressure de creasing pulse pressure and lethargy The pos sibility of pericardial constriction should be borne in mind when a uremic patient with an enlarged heart suddenly develops cardiac insufficiency

Increasing heart size with disappearance of the normal cardiac contour was demonstrated roent genologically in four of our patients during the days immediately preceding the development of manifest cardiac tamponade Repeated chest films are thus a helpful and important measure in the control af patients with uremic pericarditis Char acteristic electrocardiographic changes may be an aid but they are not constant and give no warn ing of threatening or manifest cardiac constric tion In addition measurement of the venous pressure should be a part of the routine control of patients with uremic pericarditis The use of ultrasound in the diagnosis of pericardial effusion has recently been described (10) This new tech nique seems to make rapid diagnosis possible without discomfort to the patient

In most cases uremic pericarditis provides an indication for hemodialysis however universal heparinization in connection with dialysis has been mentioned as a causative factor in the de velopment of hemopericardium (1 2 3 8 9) Coagulation studies in our patients showed a prolonged plasma thrombin time which became normal on the addition of toluidine blue This abnormality can be seen with heparin and heparin like anticoagulants as well as in uremia True hemopericardium was not seen in our patients

One patient developed an acute constriction syndrome during his first hemodialysis On peri cardiocentesis 1360 ml sero-sanguinous fluid was removed and the clinical state of the patient im proved Thereafter 16 hemodialyses with universal heparinization were carried out with no complica tions (Table IV case 1) Six other patients with uremic pericarditis were treated with intermittent hemodialysis and universal heparinization In one of these six patients pericardiocentesis had been performed twice because of a sero sanginous pericardial effusion with cardiac constriction (Table IV case 6) Hemodialysis was uncom plicated in all of these cases and in three pa tients the size and shape of the heart became normal All seven patients received kidney trans plants (Table IV) In this series eight patients with uremic pericarditis including two with cardiac tamponade have received renal trans

plants Seven of these patients are alive one 3 years post transplant

Universal heparinization in connection with hemodialysis does not therefore seem to be contraindicated in patients with uremic pericarditis

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STUDIES ON FATTY ACID METABOLISM IN DIABETICS DURING EXERCISE

VII *Plasma Glycerol Concentrations in Juvenile Diabetics during Exercise before and after Adequate Insulin Treatment*

Sven Carlstrom

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Abstract Male newly diagnosed diabetics of the juvenile type have been examined with a short exercise test on a bicycle ergometer and the plasma concentration of glycerol was followed. As earlier shown the exercise induced a greater lipid mobilization in the diabetics in comparison with control subjects studied earlier

The exercise test was then repeated after a period of adequate insulin therapy At the second examination the diabetics showed a clear tendency to normalization of the plasma glycerol curve indicating that insulin therapy will decrease the abnormally high exercise induced lipid mobilization in diabetes mellitus

Male juvenile newly diagnosed diabetics show during exercise a greater and longer persisting lipid mobilization than control subjects of the same age This is revealed by higher concentrations in plasma both of free fatty acids (FFA) and of glycerol during and after the exercise period (2 3) Adequate insulin treatment of the disease leads to a normalization of the plasma FFA concentrations (4)

The present study was undertaken to find out if insulin therapy also brings about a normalization of the plasma glycerol concentrations in connexion with exercise It is now well known that the plasma glycerol concentration is a better parameter of the lipid mobilization rate than plasma FFA as glycerol is not reutilized in adipose tissue for metabolic purposes (7 10) For that reason it might be of interest to know whether the plasma glycerol level during exercise is normalized in diabetics after insulin therapy and thus whether the normalization of the FFA curve is really due to a decrease of the lipid mobilization rate

MATERIAL AND METHODS

Five male newly diagnosed juvenile diabetics, aged 19–28 years were selected for the study All were diabetics of the juvenile onset type with subjective symptoms Some clinical data of the patients are summarized in Table I None of the patients were dehydrated or ketoacidotic when examined

The diabetic subjects were all examined in the fasting state and at the first examination none had received insulin therapy They were then treated with long acting insulin and re-examined when they clinically were under good control

The examination started in the morning After resting for 1 / hour they performed graded exercise on a bicycle ergometer at a work load of 600 kpm/min for 10 min in the supine position They were then again allowed to rest

Blood samples were taken through an indwelling arterial catheter at intervals indicated in Table II When not in use the catheters were filled with physiological saline Details concerning the examination has been published previously (2)

Plasma glycerol was determined according to Laurell and Tibbling (8) The plasma FFA analyses were made according to Trout et al (11) and the blood glucose concentrations according to Marks (9)

In addition some hemodynamic data were registered but these will be published elsewhere

RESULTS

The plasma FFA and the blood glucose concentrations during both examinations are summarized in Fig 1 At the first examination the FFA pattern during exercise is in good accordance with earlier published results (2) At the second examination performed after adequate insulin treatment there is a clear tendency to normalization

Table I *Some clinical data for the diabetic patients examined*

Case	Age (y)	Height/weight (cm kg)	Plasma creatinine	Blood pressure	Duration of symptoms (weeks)	Complications
D 1	25	177 47 5	0 8	115/75	8	0
D	19	179 65 0	1 10	130 75	3	0
D 3	[illegible]0	184 67 4	1 10	125 90	4	0
D 4	[illegible]	177 63 1	(NPN 36)	140 80	4	0
D 5	[illegible]8	183 75 0	(NPN 30)	145 90	9	0

Non protein nitrogen

Table II *Plasma glycerol concentrations (μmoles/l) during the experiment before insulin treatment*

	Time																
	Hours				Minutes												
Case	−2	−1½	−1	−½	1	3	5	8	11	13	15	18	25	40′	55	70	100′
D 1	84	76	84	114	170	138	155	175	215	215	210	190	134	90	103	1 0	1[illegible]8
D [illegible]	76	87	94	136	148	161	190	238	269	272	286	262	182	83	77	100	107
D 3	43	45	75	110	112	107	135	212	303	318	348	364	346	129	68	37	5[illegible]
D 4	84	75	105	99	100	107	154	[illegible]78	368	404	406	387	248	80	38	31	71
D 5	70	49	103	140	190	210	253	308	376	377	384	361	225	96	55	56	108
Mean	71	66	92	1 0	134	145	174	242	306	317	327	313	227	96	68	69	93
S.E.M.	7 6	8 [illegible]	5 7	7 9	16 1	19 3	[illegible]0 9	23 5	30 3	34 4	35 6	37 6	35 5	8 8	10 9	17 6	13[illegible]

Table III *Plasma glycerol concentrations (μmoles/l) during the experiment after insulin treatment*

	Time																
	Hours				Minutes												
Case	−[illegible]	−1½	−1	−½	1	3	5	8	11	13	15	18	[illegible]5	40	55	0	100
D 1	54	46	46	53	85	87	91	99	106	10[illegible]	101	101	10[illegible]	99	94	91	—
D 2	55	50	61	86	118	178	138	130	138	135	128	118	97	78	72	75	—
D 3	73	4	89	63	70	73	92	—	158	—	176	165	155	101	55	58	—
D 4	76	46	59	84	101	111	160	[illegible]56	360	387	397	374	232	88	48	46	59
D 5	51	50	77	9[illegible]	105	139	166	189	2[illegible]	[illegible]35	[illegible]35	215	159	71	39	34	64
Mean	62	47	66	6	96	108	129	169	197	215	707	195	149	87	62	61	67
S.E.M.	5.3	1.5	7 5	7 5	8 3	12.3	16 2	34 6	44 9	64 0	5[illegible]6	49 1	[illegible]4 4	5 8	9 7	10 1	[illegible]4

of the FFA curve which agrees well with earlier published findings (4) The plasma FFA curve at the second examination is not different from that found in control subjects of the same age (2)

The plasma glycerol concentrations at the two examinations are given in Tables II and III and all values are summarized in Fig 2 Compared with that of controls (3) the plasma glycerol curve at the first examination is elevated before during and for a period after exercise This is in agreement with earlier findings (3) At the second examination each diabetic shows a tendency to normalization of the plasma glycerol curve and the mean curve for the diabetics is clearly lowered However the mean curve is still somewhat elevated in comparison with that of controls studied earlier (3)

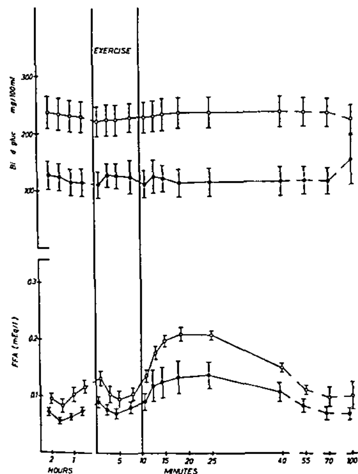


Fig 1 Plasma FFA and blood glucose concentrations before (O) and after (●) insulin treatment Mean ± standard error of the mean.

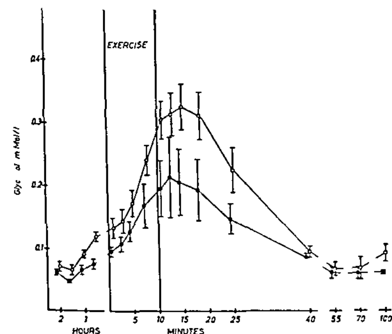


Fig 2 Plasma glycerol concentrations before (O) and after (●) insulin treatment.

DISCUSSION

One of the striking features in the deranged fat metabolism in diabetes mellitus is the increased lipolysis in adipose tissue and the consequently augmented mobilization of fatty acids and glycerol to plasma As stated in the introduction glycerol cannot be reutilized in adipose tissue and thus is the best indicator of the rate of the lipid mobilization (7 10) It is now well known that the plasma glycerol concentration is elevated at rest in diabetics (3 5) Exercise stimulates the lipid mobilization and leads to higher plasma concentration of glycerol in control subjects (1 6) and to abnormally high plasma levels in untreated diabetics (3) This is presumably due to a more lightly initiated lipolysis in the adipose tissue in diabetes Judging from the present study adequate insulin therapy appears to decrease the abnormally high exercise induced lipid mobilization in diabetes mellitus

ACKNOWLEDGEMENTS

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ACCIDENTALLY TRANSMITTED INFECTIOUS MONONUCLEOSIS

Report of a Case

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Abstract A case report is given of infectious mononucleosis (IM) accidentally transmitted to a patient by transfusion The donor two days after giving 450 ml citrated blood experienced malaise and IM was diagnosed with a typical clinical and haematological course. The citrated blood was stored for three days before the donation. The transfused patient three weeks later developed a febrile syndrome with general adenopathy and the haematological features of IM The diagnosis of IM was supported by a positive horse-cell slide agglutination test (EMNI test)

By definition infectious mononucleosis (IM) is a subacute or acute infection which is characterized by fever and a distinctive lymphocytosis and frequently by enlargement of cervical and other lymphatic glands or by a sore throat or both (25)

The etiology of IM is unknown Recently a virus called the Epstein Barr (EB) virus has been discovered in patients with mononucleosis it is a herpes like virus that has been associated with Burkitt s lymphoma cells (12) In each of 29 patients with IM the development of antibodies against EB virus was demonstrated by Niederman et al (17) by an indirect immunofluorescence test with a virus bearing cell line (EB 3) derived from a Burkitt lymphoma These antibodies absent in pre illness serum specimens usually appeared early in the disease rose to peak levels within a few weeks and remained at relatively high levels during the convalescense These and other observations indicate that EB virus or a closely related one is the etiologic agent of IM The etiology of the infection however is still a subject of debate (6 7)

IM though it has been known previously by other names was considered to be a curiosity when Sprunt and Evans (23) first coined the name almost 50 years ago There is evidence that the incidence of the disease is increasing (24) and today it is really a common disease At present IM appears to be less epidemic in character and more sporadic Transmission is probably by direct presumably intimate contact with infected persons (14)

Attempts to transmit the disease to laboratory animals by various inocula have met with negative or equivocal results Human volunteers transfused with blood from patients with IM have on a few occasions developed suggestive clinical evidence of the disease Only in a single case has an obviously successful experimental transmission been carried out in this way (28) It is noteworthy that the literature describes only two cases in which a patient accidentally acquired IM as a consequence of a blood transfusion from a donor with this disease (3 18) This statistic appears singularly unimpressive in view of the incidence of the illness

We have had a patient in whom IM occurred after a blood transfusion from a donor who experienced the first symptoms of IM two days after the transfusion

CASE REPORTS

Patient 1

L S B a 21 year-old female student was admitted to the Medical Department Sentralsykehuset Akershus on March 13 1968 She was previously healthy and had been a blood donor for one year She had last given blood on February 6 of the same year During the very first days of March she noticed oedema of the eye lids

and experienced a gen ral malaise nausea, anorexia and severe headache

Status on admission March 13 1968 General condition good. Temp 38 2 C The patient complained of headache slight anginous throat trouble and fatigue There was no rash or jaundice The examination revealed moderate reddening of the pharynx without palatal enanthema. Along both sterno-cleido-mastoids in each axilla and inguinal region a number of enlarged lymph nodes were present The lymph nodes ranged from about bean up to hazel nut-size

Internal o gans Slightly epigastric tenderness was noted during palpation of the abdomen The sp een was easily palpable two fingerbreadths below the left costal margin not tender Liver not palpable

Laboratory fi dings on adn ission were as follows Urine and spinal fluid no abnormality Hb 11 8 g 100 ml white cell count 16 700 mm with 78% atypical lymphocytes. They were mostly classed as Type III by Downey (4) Blood platelets 147 000 mm ESR mm/h serum glutamic pyruvic transaminase (SGPT) 300 units alkaline phosphatase 10 8 *mM* (Bessy Lowry) units

The horse-cell slide agglutination test (EMNI test) was positive The tests for sheep red ce l agglutinins according to Paul and Bunnell were positive and showed a titre of 1 768 in sera after absorption with guinea pig kidney Wasserman Kahn and M B R. II tests were negative

A d agnosis of IM was made by the characteristic clinical and laboratory features. The hospital course was uneventful the patient was discharged on March 21 1968 and later followed up for half a year There was a typical clinical and haematological course

During her stay in hospital we checked the information given by Patient 1 that she had donated blood two days before she experienced the first symptoms of her illness By coincidence the transfusion turned out to have been carried out in the Surgical Department of our hospital The transfused patient is hereafter referred to as Patient 2

Patient 2

M A a previously healthy dressmaker aged 18 had been admitted to the Surgical Department on March 1 due to a traffic accident

Status on admission Ma ch 1 1968 The patient complained of pains in the chest and in her left thigh Further examination revealed fractures of the left clavicle and the tenth right rib and some laceration of the soft tissue of the left thigh. The temperature was normal There was no adenopathy

Internal organs The liver and the spleen were not palpable

Labo atory f ndings on admission were as follows Hb 10 7 g/100 ml white cell count 6 00 mm with 16 % lymphocytes, 2% monocytes, and the 82 granulocytes showed a moderate shift to the left"

On her first day in hospital she received 4 0 ml citrated blood from Patient 1 who we learnt 14 days later had infectious mononu leosis. Thus the blood had been stored for three days before the transfusion

On March 20 Patient 2 was more closely examined in the Medical Department She was at that time running a variable pyrexia with temperature from 37 7 to 38.2 C She complained of general malaise and a tired feeling which could be a result of the accident She had, however had a mild sore throat for two days and on examination pharyngeal injection without exudate was found There was no eyelid oedema no exanthema no palatal enanthema General adenopathy was present. Of the cervical glands the posterior chains were palpable down to the clavicle the individual glands were of the size of beans The edge of the liver cou d b felt below the right costal margin non tender The spleen was not palpable

Labo atory investigations On March 20 the percentage of Hb was 12 3 g/100 ml ESR 43 mm h white cell count 12,400/mm with 42 neutrophils 58% mononuclear cells Most of the mononuclear cells were atypical lymphocytes (43 % of the total number of white cells) The abnormal cells we encountered were mostly classed as Type I and II by Downey a few cells with the appearance of Type III were also seen Serum g utamic pyruvic transaminase (SGPT) was 22 units thymol 1.6 Maclagan units

The horse-cell slide agglutination test (EMNI test) was distinctly positive and was repeated on March 27 and again found indisputably positive The tests for sheep-cell a glutinins according to Paul and Bunnell were negative and remained so throughout the time of observation

Tests for toxoplasmosis the Brucella agglutination test, and tests for cytomegal virus were all negative Wasserman Kahn and M B R II test were also ne ative

The clinical picture gradually improved On April 1 the patient had no complaints which unambiguously could be referred to the transmitted IM The fatigue however was still present. The liver cou d not be felt the general adenopathy was easily demonstrated although in recession

On April 1 examination of the peripheral b ood revealed a relative lymphocytosis of 41 The number of atypical lymphocytes amounted to 24% of the total number of white cells (6100 mm³) cells of Downey Type I were now dominant. The EMNI te t was now negative

Blood films examined throughout the month of April showed a decreasing number of mononuclear cells. In most of the blood films, cells were seen which were intermediate in morphology between normal lymphocytes and the atypical cells, and it was therefore possible to estimate only the approximate proportion f atyp cal mononuclear cells in the total white population. The range of atypical cells was 610 to 1100 mm and the proportion of these cells in the total lymphocyte count averaged —% In July the blood picture was normalized

DISCUSSION

Many attempts have been made to transmit IM to animals and humans inoculated with whole blood serum excised lymph nodes faeces and gargle washings from patients with the disease

(1 8 9 16 27) Convincing evid nce of success ful transmission of this disorder has b~en missing in almost all these experiments Two attempts only may be acceptable as instances of successful transmission to man—that of Sohier et al (21) and particularly that of Wising (28) In a critical review of transmission experiments with IM Hoagland states that he is inclined to believe that Wising s experiment can be regarded as suc cessful" (14) In both cases the experimental trans mission of IM was attempted with whole blood as inoculum

Accidental transmission of IM by blood trans fusions seems to be very rare as only two cases are reported in the literature (3 18) De Vos and Kuipers (3) in 1951 reported that they involuntar ily transmitted IM by transfusing 400 ml of blood into a patient The latter experienced fever 21 days later splenic enlargement was observed and lymphocytes constituted 88 % 42 days after trans fusion Many atypical lymphocytes were present Thirty four days after transfusion the heterophil antibody titre was 1 128 The donor one week after giving blood experienced malaise and an examination revealed a mononucleosis Paloheimo and Halonen in 1965 (18) described a mono-nucleosis like syndrome in a 15 year-old girl with congenital heart disease The patient received a blood transfusion from pooled blood of nine blood donors extracorporal circulation was not used Five to six weeks later the typical" syn drome with general malaise fever lymphadeno-pathy lymphocytosis with atypical lymphocytes occurred The Paul and Bunnell test was nega tive One of the blood donors had had IM

Circulating atypical mononuclear cells often unassociated with fever and lymphocytosis have been observed in the postoperative period in pa tients who have had open heart surgery Forster (10) reported these findings in 30 of 180 patients It has been suggested that post transfusion mono-nucleosis in such cases may represent an immune response to viable lymphocytes contained in freshly donated blood essentially a graft of liv ing lymphocytes (5) In some cases however it has been proven that the patient accidentally has acquired a cytomegalovirus infection (for review see Paloheimo et al (19))

Our patient acquired an illness with the clinical criteria of a mild attack of IM about three weeks after having received a transfusion with 450 ml from a donor who two days after the donation experienced the first symptoms of typical IM We regard the illness of our patient to be a case of post transfusion mononucleosis due to trans mission of the (unknown) causative agent of IM A rise and fall in abnormal lymphocytes was ob-served during the illn ss and a repeated horse cell slide agglutination test (EMNI test) was indisput ably positive 20 and 22 days respectively after the transfusion The EMNI test however was negative five weeks after the transfusion

The incubation period of naturally acquired IM is considered to be about 33–49 days (13) A case report of IM occurring 21 days after blood transfusion shows that the incubation pe riod might be shorter when the disease is trans mitted in this way (3) This last experience is in accordance with the interval between the trans fusion and the state of illness in our patient

Although the presence of abnormal lymphocytes is a characteristic finding in IM it is not patho-gnomonic Numerous atypical lymphocytes are frequently observed in infective hepatitis and similar cells have also been described in brucel losis acquired toxoplasmosis cytomegalovirus in fection and all rgic conditions (for review see Penman (20)) A diagnosis of infective hepatitis could be excluded in our case On the contrary the normal liver function tests as in our patient are rather unusual in patients with IM By the improved tests now available it has b en shown that the function of the liver is impaired in 90 to 100% of patients with IM We have however occasionally observed patients with (seropositive) IM in whom serial blood samples drawn had a normal amount of serum glutamic pyruvic trans aminase (SGPT) This experience is in accordance with that of Hoagland (14) though we admit that an increase of this serum enzyme is the rule during the course of IM In a recent report by Trostman and With (26) the thymol turbidity test and the serum glutamic-oxaloacetic transaminase (SGOT) were found to be positive in the majority but not in all cases of IM during the acute febrile stage

Acquired toxoplasmosis brucellosis and cyto-megalovirus infection could be excluded in our patient

Until the aetiology of IM is ultimately defined its diagnosis must necessarily be based on a com posite of clinical haematological and serological

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ATRIAL TACHYCARDIA WITH BLOCK

A Clinical Study of 40 Episodes in 32 Patients

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Abstract A study has been made of 40 episodes of atrial tachycardia with block in 32 patients observed during the years 1965 through 1968

Mitral valve disease was the most frequent underlying disease while aortic valve disease was nearly absent The rhythm disorder produced significant deterioration in a majority of the patients and precipitated severe complaints even in persons with a normal heart Most episodes ran a continuous course the paroxysmal variety bein rather infrequent It is therefore suggested that the arrhythmia is termed atrial tachycardia with block and the prefix "paroxysmal" added only when distinct attacks are observed.

Only three patients died all from their underlying disease This mortality is probably the lowest ever recorded in series of this rhythm disturbance

The diagnostic criteria largely proved satisfactory but differentiation against atrial flutter remains controversial

Digitalis poisoning was possibly present in only nine of the 40 episodes Contributory causes including hypokalemia were not demonstrated. Nor was any specific heart rhythm found to be the prime predecessor of atrial tachycardia with block

Treatment consisted of discontinuance of digitalis and peroral administration of potassium chloride Some 3–4 days thereafter electroconversion was undertaken. This treatment proved to be safe reliable and effective and superior to treatment with various antiarrhythmic agents

Atrial tachycardia with block is easily overlooked but important to recognize Firstly because it often produces serious clinical deterioration Secondly because it may be misinterpreted as atrial flutter which calls for increased dosage of digitalis Atrial tachycardia with block however may be a sign of digitalis poisoning and a further increment thus lead to disastrous results (7 10) Thirdly atrial tachycardia with block is unique in being able to present as a bradyarrhythmia

This report is a study of atrial tachycardia with block its diagnostic criteria its varying clinical pictures and its relationship to treatment with digitalis glycosides

MATERIAL

The series comprises 40 episodes of atrial tachycardia with block in 3_ patients admitted during the years 1965 through 1968 This gives a mean number of ten episodes discovered among some 7000 ECGs yearly The frequency did not rise during the observation period The arrhythmia occurred on three separate occasions in two patients, and on two separate occasions in four patients

The diagnosis of atrial tachycardia with block was made according to the criteria put forward by Lown et al (10) an atrial rate between 150–_50 per min, an isoelectric baseline between the P waves in all leads and the existence of atrioventricular block beyond simple prolongation of the P R interval

An important diagnostic aid in this study was obtained from the esophageal lead the use of which caused minor discomfort even in critically ill patients

In this series atrial tachycardia with block (AT with block) did not consistently appear in paroxysms Consequently the prefix paroxysmal" has been omitted in the following.

RESULTS

The total series (Table I) showed mitral valvular disease to be the prevalent underlying disorder while the absence of aortic valve disease was unexpected as these two disorders are evenly distributed at the department

Among the five men with other heart diseases were the only one with aortic valve disease another treated with closure of a secundum type of atrial septal defect one with an isolated pulmonic incompetence one with a sino-atrial and

Table I. *Distribution of sex, age and underlying disease in the series of atrial tachycardia with block*

Sex	No.	Mean age	Mitral valve dis.	Atheroscl. heart dis.	Other heart dis.
Men	12	61	1	4	5
Women	20	53	11	2	6
Total	32	56	12	6	11

Table II. *Frequency distribution of atrial and ventricular rates in 40 episodes of atrial tachycardia with block*

Rate per min	Atrial	Ventricular
<100	—	19
100–149	3	20
150–199	13	1
200–249	20	—
>250	4	—
Mean	213	105

one with a complete atrioventricular block. The latter two both required treatment with an artificial pacemaker.

Among the corresponding six women there were two who had been operated on for atrial septal defect of secundum type, one suffered from amyloidosis, one from a sino-atrial and one from a complete atrioventricular block, while one suffered from a thyretoxic cardiomyopathy.

In two patients, one of each sex, no underlying disease could be demonstrated.

The heart volume was normal in seven patients and considerably enlarged in 18. In the remaining seven patients the heart was markedly enlarged, the calculated heart volume exceeding 1000 ml per sq.m. body surface.

The clinical effects produced by AT with block usually were apparent. A majority of the patients worsened, sometimes critically, when under the influence of the arrhythmia. Its adverse effects could best be assessed by the clinical improvement following its abolition.

This study showed AT with block to be a rhythm disorder which in principle followed a continuous course. Only three patients experienced distinct bouts or paroxysms. In the remainder AT with block tended to persist for weeks, months or years until interrupted by treatment. One woman in this series suffered from the arrhythmia continuously for six years. In such long-standing cases the atrioventricular block may vary and produce different heart rates. This variation is, however, not to be mistaken as attacks.

Only three of 32 patients died in the present series, the mortality thus being unusually low. A 37-year-old woman died from amyloidosis. Another woman aged 62 with advanced mitral valve disease had attacks of AT with block accompanied by severe anginal pain. Discontinuance of digitalis caused disappearance of the arrhythmia, intermediate occurrence of nodal rhythm and ultimately of sino-atrial block requiring treatment with artificial pacemaker. The patient died during an attack of anginal pain when under preoperative care for implantation of an artificial mitral valve. Necropsy revealed a calcified mitral ostium, coronary artery obstructions and fibrosis around the conduction system. A man aged 58 died from advanced atherosclerotic heart disease.

The above patients all died from their primary disease, but the rhythm disorder seemed to add markedly to the deterioration leading to death.

The diagnostic criteria proved largely satisfactory (Table II). The highest and lowest atrial rate recorded was 112 and 273, respectively. Nearly half of the episodes had ventricular rates

Table III. *The frequency of various types of atrioventricular block in 40 episodes of atrial tachycardia with block*

Type of A-V block	No.
2:1	2[illegible]
3:1	6
4:1	2
Wenckebach	3
Varying	4
Complete	3

Table IV. *The rhythms immediately preceding 23 episodes of atrial tachycardia with block*

Preceding rhythm	No.
Sinus rhythm	9
Atrial fibrillation	9
Atrial flutter	1
Varying rhythms	4

I V

25/1965 II V2

III V3

AVR V

AVL V5

AVF V6

Atri l rate 230/min Ventr rate 120/min

R-R: o 50 o 50 o 44 o 60 o 50 o 50 o 44 o 60

E ophag lead

P P P AVL P P P P P P P P P P P P P

Fig 1 Case 1 Upper tracing shows the ECG interpreted as sinus rhythm with premature atrial beats. Lower tracing illustrates the esophageal lead with a peculiar second degree block

below 100 per min The figures for mean rate indicate 2 1 block to be prevalent

An additional diagnostic criterion of considerable merit is the varying P P intervals which were noted in many episodes of the present study This variation is in sharp contradistinction to th perfect regularity seen in atrial tachycardia without block and in atrial flutter

Carotid sinus pressure as a diagnostic aid was not consistently applied but definitely proved less reliable than in atrial flutter

Usually the P waves are best seen in lead V_1 but often may be impossible to detect The superior diagnostic tool is then an esophageal lead which proved to be the most useful and accurate adjunct in this study

Still differentiation against atrial flutter may prove difficult especially at atrial rates around 270 per min where the two rhythms may overlap Often those characteristics of atrial flut er such as baseline oscillations and P wave negativity in leads II III and aVF may be impossible to ascertain (1)

The atrioventricular block comprised a l the common varieties (Table III) and an add ional type which could not be classified Some cases of this particular block showed some kind of pe riodicity over a longer range of heart beats The pulse rate in such cases was so irregular as to give a perfect imitation of atrial fibrillation

In some patients the second degree block was observed to disappear in response to exercise and to general anesthesia The administration of atropine likewise abolished the block in one case

Among the three cases with complete block the latter disappeared in one man after successful electroconversion In another man the same procedure restored sinus rhythm in the atria but the block persisted A woman suffered from AT with block and recurrent syncopes Electroconversion was ineffective and an artificial pacemaker wi h fixed rate was required A year later the patient was readmitt d with tachycardia due to interferen e rhythm The complete block had then reced d to a se ond degree 2 1 blo k

Dig ta medication or poisoning as a possible etiologic fa tor in AT with blo k was given special attention Only nine episodes could more or less safe y be at ributed to an excess ingestion of d gi alis glyco ides In 22 epi odes digitalis had

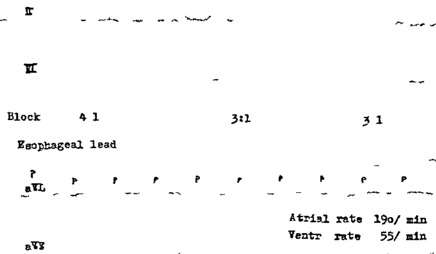


Fig 2 Case 2 This ECG was initially interpreted as sinus bradycardia but the esophageal lead disclosed atrial tachycardia with 3 1 and 4 1 atrioventricular block and the corresponding low ventricular rate A low pulse rate does not exclude the presence of atrial tachycardia with block

been used in constant dosage for months or years when AT with block appeared for unknown reasons A total of nine episodes were without relationship to the use of digitalis

Slight hypokalemia was observed in one pa t only Other contributory causes such as trauma copious diuresis concomitant disease or grave clinical deterioration (10) could not be demonstrated

The rhythm present at the outbreak of AT with block could be safely determined in 23 episodes (Table IV) It seems clear that no specific rhythm can be regarded as the prevalent predecessor of AT with block

The treatment of AT with block in this study consisted in immediate discontinuance of digitalis and peroral ingestion of potassium chloride Electroconversion was then to be done 3–4 days later This approach covered a twofold purpose Firstly a possible digitalis poisoning was allowed to disappear Thus spontaneous conversion to sinus rhythm occurred in six episodes in the course of 2–3 days Secondly the use of various drugs with several unwanted side effects was avoided Instead the patients were exposed to the safe simple and effective procedure of electroconversion Thus 33 episodes were successfully converted while only one episode proved resistant to this treatment

In some cases of possible digitalis poisoning potassium was administered intravenously without the immediate effect noted by others (4 10) Other antiarrhythmic agents were given a trial also but none of them proved consistently effective

It is to be added that the great majority of the patients treated were dismissed on continued digitalis So far the rate of relapse has been negligible

CASE REPORTS

In the sequel some illustrative case histories from patients suffering from AT with block are presented

Case 1 woman 57 years old (Fig 1) In the last six years palpitations accompanied by dyspnea severe angina pectoris and syncopes Treated with digitalis for five years without effect Digitalis discontinued 13 months prior to the admission The first ECG (8 8 1968) was interpreted as sinus rhythm with atrial premature beats, but close inspection aroused suspicion of the presence of AT with block which was verified by means of an esophageal lead A peculiar repetitive second degree block could be observed Further clinical examination did not reveal coexistent or underlying disease Sinus rhythm was restored by means of electroconversion and the patient dismissed on quinidine treatment.

Case 2 man 50 years old (Fig 2) For two years severe angina pectoris treated with digitalis anticoagulants and nitroglycerine without effect On admission the first ECG (11 4 1966) was initially interpreted as sinus bradycardia However an esophageal lead disclosed an AT with block with a remarkably low ventricular rate Although no signs of digitalis poisoning were present, this agent

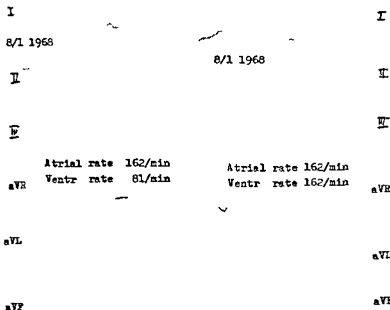


Fig 3 Case 3 The left tracing shows atrial tachycardia with regular 2 1 block, which in the right tracing disappears in response to an exercise test

was discontinued After three days spontaneous conversion to sinus bradycardia took place Further clinical examination gave no evidence of underlying disease Coronary heart disease could not be demonstrated by objective means The heart was of borderline size The patient was dismissed on treatment with digitalis and quinidine

Case 3 woman 21 years old (Fig 3) In the last year troubled by attacks of uncomfortable palpitations accompanied by pain fear and dizziness Clinical examination was unrevealing The ECG during attacks showed AT with block interrupted by periods of sino atrial block and periods of atrial fibrillation During AT with 2 1 block a Master s two step test was done The block thereby vanished Digitalis quinidine and beta adrenergic blocking agents failed to prevent the bouts of AT with block.

Case 4 woman 46 years old (Fig 4) About four weeks after mitral re-commissurotomy sudden deterioration with dyspnea and chest pain ECG (11 6 1964) revealed AT with block most distinctly seen in V This block exhibited Wenckebach s periods Sinus rhythm was

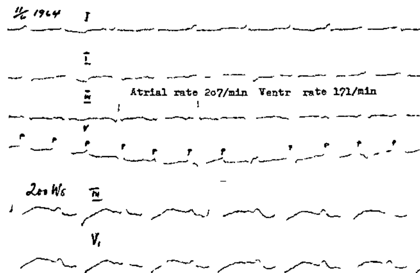


Fig 4 Case 4 Upper tracing shows atrial tachycardia with distinct, small P waves and isoelectric baseline intervals in lead V The block exhibits Wenckebach periodicity Lower tracing demonstrates sinus rhythm obtained by electroconversion Note the identical shape of P waves in the two tracings

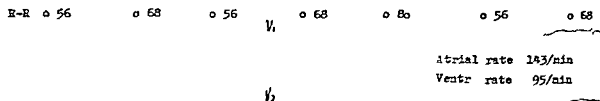


Fig 5 Case 5 Atrial tachycardia with second degree block of a peculiar appearance with repetitive periods unlike those of Wenckebach.

restored by means of electroconversion. Note the resemblance of the P waves in the two rhythms This should be so because the impulse focus in AT with block is known to lie in the near vicinity of the sinus node (1)

Case 5 woman 57 years old (Fig. 5) Operated upon with transplantation of an artificial mitral valve Some weeks after surgery occurrence of AT with block This block showed a peculiar periodicity over five beats alternating with shorter periods of 2 1 and 1 1 block Discontinuance of digitalis, treatment with potassium, quinidine procainamide and electroconversion failed to abolish the arrhythmia.

Case 6 woman 69 years old (Fig. 6) For some years treated for paroxysmal atrial flutter Admitted for troublesome tachycardia on March 29 1966 The ECG revealed signs typical of AT with 2 1 block and was described accordingly Sinus rhythm was restored by means of electroconversion and the patient dismissed on continued digitalis Readmitted on November 14 1966 for tachycardia The ECG showed atrial and ventricular rates identical with those of the ECG from March 9 However this time the ECG was interpreted as atrial flutter This was due to the presence of negative P waves and absence of isoelectric baseline in lead V although typical flutter oscillations were nowhere to be seen in the whole ECG Again sinus rhythm was restored by direct countershock.

Comparison of lead V_1 of the three ECGs shows the P waves on March 29 to be very like those produced by the sinus node on November 18 while those from November 14 are different from the latter

All these findings would support the initial diagnoses of AT with block on March 29 and atrial flutter on November 14 Experimental data indicate that the ectopic focus in AT with block lies in the superior part of the atrium near the sinus node (1) In contrast, the ectopic focus in atrial flutter is located low in the atrium

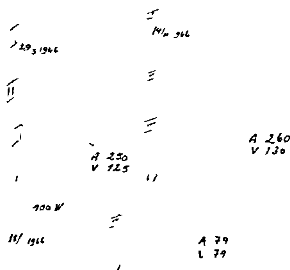


Fig 6 Case 6 *The upper tracing shows two ECGs from* the same patient on various occasions The atrial and ventricular rates are identical. The left tracing was interpreted as atrial tachycardia with block, the right tracing as atrial flutter Lower tracing shows sinus *rhythm obtained by means of electroconversion.*

COMMENTS

This study provides some additional information on atrial tachycardia with block Firstly it makes

clear that this arrhythmia is not confined only to elderly patients with serious heart disease (4 10) but also occurs in young patients and in persons without demonstrable disease at all Mitral valve disease was the prevalent underlying disorder in the present series For unknown reasons aortic valve disease showed a corresponding absence

Secondly this series showed AT with block to appear mainly in a continuous form and seldom in attacks Similar observations have been made by others (11) and a case of 25 years continuous duration has been reported (12) It is suggested that the prefix paroxysmal" in AT with block should only be used in the presence of distinct attacks

It is obvious from this study that the adverse effects produced by AT with block cannot be ascribed to a high ventricular rate (11) because half of the episodes showed ventricular rates below 100 per min Additionally a man with a pulse rate of 55 suffered from severe angina pectoris Probably the atrial rate and the inefficacy of the atrial systole play a greater role in the production of symptoms

The mortality in the present report was less than 10% in contrast to figures from 28 to 58% in other series (4 6 10 11) Deaths due to digitalis poisoning were not encountered but have been noted by others (7 10)

The diagnostic criteria for AT with block as laid down by Lown et al (10) provide a good coverage of the subject Still controversy may arise from differentiation between AT with block and atrial flutter It seems wise to regard a diagnosis of AT with block as dubious if the atrial rate exceeds 270 per min even when all diagnostic criteria are satisfied In practice this differentiation is not vitally important The problem is easily solved by discontinuing digitalis and applying a direct countershock some days thereafter This treatment is equally effective in both arrhythmias

The present study also revealed a type of block not previously described with repetitive periods over a longer range of heart beats Furthermore it showed the second degree block to disappear in response to exercise and general anesthesia This may explain that the presence of a continuous type of AT with block may pres n with intermittent bouts which virtually are f.l e and are due only to abolition of the atrioventricular block in response to exercise or augmented sympathetic stimuli of other origin

Coexistence of sino atrial block and AT with block has been observed in a case reported by Laake (9) In this series two similar cases were seen one of them being so serious that implantation of an artificial pacemaker became necessary

The etiology of AT with block is unknown but poisoning with digitalis has been regarded as a chief cause in published series (4 6 10) This view has been disregarded by some authors who have noted only beneficial effects from treating all cases of this rhythm disorder with digitalis (8 11) According to the present study the etiologic role played by digitalis has probably been overestimated in the past. However digitalis poisoning has always to be considered as further poisoning has had disastrous effects (7 10) On the other hand prolonged withdrawal of digitalis is to be avoided because a majority of patients suffering from AT with block also suffer from heart failure

Both these requirements are met by the treatment of AT with block as employed in the present series Discontinuance of digitalis administration of potassium chloride by the oral route and application of electroconversion after some few days represent the constituents of a safe simple and effective therapy It is followed by prompt reinstitution of digitalis treatment This kind of therapy has been effective where other therapy has failed (3) However treatment with diphenyl hydantoine (2) propranolol (5) quinidine or procainamide (4) may be justfied in certain circumstances

This study also proved that no specific heart rhythm preceded the commencement of AT with block If so the outbreak of AT with block could have been fores en and possibly prevented However AT with block can follow sinus rhythm as well as all the commonly occurring rhythm dis ord rs in patients with heart disease

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EFFECT OF APTIN A β ADRENERGIC BLOCKING AGENT IN ARTERIAL HYPERTENSION

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Abst act The antihypertensive effect of a new beta adrenergic blocking agent Aptin has been studied in a group of 1? patients with moderate arterial hypertension The trial was carried out using a conventional double blind cross-over design

A significant reduction of 18 mm Hg in the systol c blood pressure at rest was recorded for the entire group The corresponding decrease of 7 mm Hg in the d stolic pressure was not signif cant During exercise a more pro nounced reduction was recorded than at rest The cor relation found between the reduction in systolic blood pressure and heart rate during exercise during the Aptin treatment was probably significant

The antihypertensive effect of Aptin was considered as moderate The drug may be of therapeut c value in com b nation with other antihypertensive drugs No significant adverse effects or changes in results of laboratory tests were noted during the Aptin treatment

The favourable effects of beta adrenergic block ing therapy in angina pectoris and cardiac ar rhythmias have been documented in several clini cal trials

During recent years an antihypertensive effect has been indicated during such therapy in patients with arterial hypertension (3 4 5 6 8 11) The reduction of the systolic blood pressure is twice as great as that of the diastolic according to Waal (11) The antihypertensive effect of the beta adrenergic blocking agents has usually been con sidered as moderate There are different opinions concerning their therapeutic value in arterial hy pertension

The antihypertensive effect of Aptin a new beta adrenergic blocking agent with a weak sym pathomimetic effect has been studied on out patients with moderate arterial hypertension The effect of Aptin was examined on patients at rest as well as during exercise

MATERIAL

The material comprised 1? patients, six men and six women between the ages of ?1 and 55 They were all previously subjected to an examination at the Medical Department. Their arterial hypertension which was of a moderate degree had been recognized for at least 3 months and maximally for 20 years The clinical d agnoses are given in Table I Case K E. had a stenosis of his right renal artery without any demonstrable reduction of the renal function With one exception all had hyper tensive eye ground changes Some anthropometric data are given in Table I

METHOD

The trial was carried out using a double-blind cross-over design The systolic and diasto c blood pressures were recorded indirectly The blood pressure cuff was applied on the patient s right overarm Diastol c pressure was re corded at the muffling of the Korotkoff sounds Every second week the blood pressures were recorded in the supine position after 10 min rest After an initial control pe od of 2 weeks without treatment all patients received placebo for a further 2 weeks Thereafter followed two treatment periods of 1? weeks with placebo and Aptin respect vely in a randomized order All tablets were identical in appearance and taste and they were given four times daily Dosage of Apt n was commenced at 50 mg q d and each dose was increased by 5 mg after weeks and by another 5 mg after 4 weeks

At the end of these 12 week periods all patients per formed a standardized work test on a b cycle ergometer according to Sjostrand–Wahlund The physical workin capac ty at a pulse rate of 170 be ts min (W) was calculated in the con entional way by interpo ation or extrapolation (cf 1) Hemo lobin whole blood count,

Table I Some anthropometric and pre therapy data of 12 patients with arterial hypertension and the clinical diagnoses

Case	Sex	Age (y)	Weight (kg)	Radiological heart volume (ml/m BSA)	Fundus hypertonicus	Heart rate at rest (beats/min)	Blood pressure (mm Hg)	W_{170} (kpm/min)	Clinical diagnoses
E N	♀	49	63	440	II	93	220/130	410	Hypertonia ess benign c mb cordis
B L	♂	46	68	420	I	81	185/125	670	Hypertonia ess benign
K E	♂	50	79	390	II	95	210/125	760	Stenosis art ren dxt c hypertonia
J H	♂	44	79	490	II	54	175/95	1530	Hypertonia ess benign
E J	♀	51	52	500	I	89	180/105	870	Hypertonia ess benign
A G	♂	45	76	350	II	90	200/105	520	Hypertonia ess benign
B P	♂	43	72	520	II	70	185/120	1060	Hypertonia ess benign c mb cordis
G N	♀	54	71	350	II	100	195/105	530	Hypertonia ess benign
S L	♀	21	53	260	0	88	160/100	550	Hypertonia ess benign
B N	♂	29	65	420	I	104	155/100	580	St p coarctatio aortae oper + hypertonia ess benign
S E	♂	55	80	520	II	93	155/100	900	Hypertonia ess benign c mb cordis
V W		55	59	335	I	83	205/115	500	Hypertonia ess benign

liver function tests, electrolytes, electrophoresis, and serum-creatinine were checked for all patients during the trial Non parametric statistics were used for testing the significance of difference and correlation (9)

RESULTS

Blood pressure at rest

During the pre treatment period the blood pressure values for the entire group were 187/120 and 182/125 mm Hg respectively At the third examination when the patients had taken the placebo for 2 weeks the corresponding values were 181/109 The latter were chosen as control values

After 12 weeks on Aptin the systolic blood pressure was reduced in ten out of 12 patients and the diastolic blood pressure in seven The mean pressures were 162/104 mm Hg at the end of the period There was usually a successive reduction in the pressure during the Aptin treatment (Fig 1) No decrease in the systolic or diastolic blood pressures occurred during the placebo period as compared to the control values The mean values at the end of this period were 180/111 mm Hg

The difference of 18 mm Hg between the systolic blood pressure at the end of the Aptin and placebo periods is statistically significant ($p <$ 0 01) The corresponding decrease of 7 mm Hg in the diastolic pressure is not significant

There was no correlation between the reduction in systolic pressure during the period of Aptin treatment and the corresponding reduction in the heart rate at rest

Blood pressure during exercise

The mean systolic blood pressure during heavy exercise (mean heart rate 165 beats/min) for the entire group was 264 mm Hg at the end of the placebo period The corresponding pressure recorded during work at the same work loads at the end of the Aptin period was 236 mm Hg The difference of 28 mm Hg is statistically significant ($p <$ 0 01) During the latter period 11 of 12 patients had a reduced pressure

The correlation found during the Aptin treatment between the reduction of the systolic blood pressure and the reduction in heart rate during exercise at the same work load and in relative

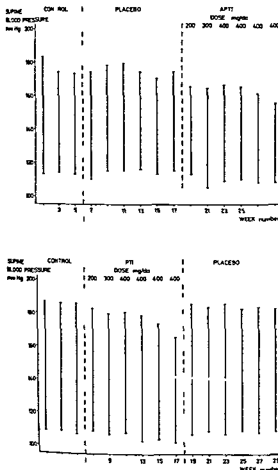


Fig 1 Mean systolic and diastolic blood pressures from a double-blind cross-over trial of Aptin in a group of 12 patients with moderate arterial hypertens on

steady state was probably significant ($p < 0\ 05$) No such correlation was noted concerning incre ments in W_{170} and blood pressure

No adverse effects that could be attributed to Aptin were reported by the patients during the trial nor were there any significant changes in laboratory findings

DISCUSSION

The reduction in the blood pressure at rest dur ing treatment with Aptin in the present group of patients with moderate arterial hyp rt ension is in magnitude similar to that found by Tibblin and Åblad (10) Further the reduction in pressure aft r Aptin is about equal in magnitude to hat reported for propranolol by Richards (6) and Waal (11)

The antihypertensive effect of Aptin was more pronounced during exercise than at rest Similar results have previously been observed in acute tests with propranolol (8)

The reduction of the blood pressure during beta adrenergic blocking therapy has been at tributed to cardiac effects of these drugs rather than to effects on the blood vessels (4 5 8 11) The correlation between the reduction in heart rate and systolic blood pressure during exercise in hypertensive patients during Aptin treatment gives further support for such an interpretation In acute tests with Aptin as well as with propranolol Johnsson (2) found that a decrease in mean ar terial blood pressure of about 10 mm Hg during beta blockade was accompanied by a decrease in cardiac output in some hypertensive patients

Furberg (1) has shown that the effect of beta blockade of the heart rate during exercise ex pressed as W_{170} was generally most pronounced in subjects with signs of a hyperkinetic circula tion and least in those with signs of a hypokinetic circulation Sannerstedt (7) found a more hyper kinetic circulation in hypertensive patients with out evidence of organic changes in the cardio vascular system than in patients with evidence of organic damage Judging from these investigations an antihypertensive effect of Aptin would be ex pected primarily in patients with signs of a hyper kinetic circulation and without evidence of organic cardiac damage

The antihypertensive effect of Aptin in patients with arterial hypertension may be regarded as moderate It is possible that beta adrenergic block ing therapy has a therapeutic value in combina tion with other antihypertensive drugs

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ANTIHYPERTENSIVE THERAPY WITH ALPRENOLOL A β ADRENERGIC RECEPTOR ANTAGONIST

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Abst act In a double-blınd cross-over study on 11 pa tients wıth hypertens on the therapeutı effect of the β adrenergıc receptor antagon st alprenolol has been com pared wıth that of placebo each preparatıon beın ad mınıstered for 12 weeks Alprenolol was admınıstered ın daıly doses of 00 mg durıng the fırst two weeks 300 mg durıng the followıng two weeks and 400 mg for the remaın n eıght weeks Compared to placebo al preno ol produced on the average statıstıcally sıgnıfıcant reductıons of about 20 mm Hg systolıc and 10 mm Hg dıasto ıc blood pressure ın supıne as well as ın standıng and sıttıng posıtıons The hypotensıve effect of alprenolol followed two dıfferent tıme courses In one group of patıents the effect was fully deve oped after the fırst two weeks of treatment In another group the effect cont nued to ıncrease throughout the twelve week treat ment perıod reach ng an average reductıon of 36 mm Hg systolıc and 23 mm Hg dıastolıc p essure At the begınnıng of treatment wıth the hıgher dos of alprenolol one patıent complaıned of attacks of effort angına probably related to a pronounced reductıon of the blood pressure Otherwıse no s de-effects or complıcatıons were observed

A reductıon of the arterıal blood pressure has been observed ın hypertensıve patıents treated wıth β adrenergıc receptor blockıng agents such as pronethalol (21 26) propranolol (13 20 22 23 31) and oxprenolol (6 7) In most studıes the β blockers were gıven ın moderate dosage and found to have an antı hypertensıve effectıveness of about the same order of magnıtude as that of the thıazıde dıuretıcs (7 13 20) However ın the most ext nsıve and long lastıng tudıes re ported Prıchard and Gıllam (22) found pro pranolol gıven ın ındıvıdualızed and often ex tremely hıgh doses to be a very actıve antı hyp r tensıve agent of at least the same potency as bethanıdıne guanethıdıne and methyldopa It ıs generally agreed that the β blockers d not pro duce postural or exertıonal hypotensıon (22) There are controversıal reports as to the tıme curve for the antı hypertensıve effect of these agents The antı hypertensıve actıon of propranolol was found to be characterızed by a gradual onset over several weeks ın one study (22) Others found that the effect was fully establıshed already after the fırst day of treatment (31)

The present study concerns an evaluatıon of the antı hypertensıve actıon of alprenolol (B taptın® Aptın®) a β receptor antagonıst for whıch thera peutı effectıveness has been demonstrated ın angına pectorıs (4 5) cardıac arrhythmıas (16) and neurocırculatory asthenıa (19) Pharmacolog ıcal studıes ın anımals ındıcate that alprenolol dıf fers from propranolol by havıng a moderate β stımulatıng actıvıty (32) and thıs property modıfıes the haemodynamıc effects of the drug ın both anımals and man (11)

The antıhypertensıve effect of alprenolol has been ınvestıgated on outpatıents wıth arterıal hy pertensıon ın two parallel double blınd studıes runnıng for seven months In one study (14) the effe t of alprenolol was examıned on patıents at rest as well as durıng exercıse whıle ın the present study the effect of the drug was studıed on patıents at rest only

MATERIAL

The trıal began wıth 14 patıents havıng supıne systolıc pressures not less than 150 mm Hg and d astol c pres u es not le than 100 mm Hg Du ın the pre t eat ment ont ol perıod b ood pressure values below these l els wer ded ın three of the patıents and th y were subsequently excluded f om the study The 11 re maınıng p t s had pre treatment supıne sys ol c pres sures betw n 155 and 200 mm Hg and dıastol c pres

Table I *Clinical information concerning investigated patients*

The symbol + for hypertensive eye ground changes indicates attenuated arterioles or calibre-changing arterioles

Pat	Age	Sex	Hypertensive eye ground changes	Heart volume ml BSA	Left ventricular hypertrophy on ECG
C	31	♀	+	340	0
K	58	o	+	470	(+)
I	61	o	+	5 0	0
A	51	o	+	410	0
B	56	^	+	410	+
AA	46	o	+	460	0
M	57	♂	+	500	+
F	30	♀	+	260	0
N	43	♂	+	—	0
L	56	♂	0	420	0
O	50	^	+	350	0

sures between 100 and 130 mm Hg Patients with bron chial asthma chronic bronchitis heart failure grade III to IV retinopathy or renal hypertension were not in cluded in the trial

A summary of the clinical information concerning the 11 patients taking part in the study is presented in Table I

METHOD

The trial was carried out on a double-blind cross-over design After an initial control period of two weeks without treatment all patients received placebo for a further two weeks Thereafter followed two 12 week treatment periods with placebo and alprenolol respectively in randomized order Alprenolol was given in a dose of 100 mg four times daily after a run in period of four weeks the daily dose being 200 mg for the first two weeks and 300 mg for the following two weeks The placebo tablets were identical in taste and appearance with alprenolol tablets

The patients attended the clinic every second week and on each visit their blood pressure was recorded with a cuff in sitting standing and supine positions after 5 min talking Side-effects were assessed by a symptom questionnaire which was read to the patient B fore the start of the trial and at the end of each 12 week period of treatment blood and urine estimations were carried out to detect any possible toxic effect of the drug The following variables were studied haemoglobin white blood corpuscle count reticulocytes thrombocytes SGOT SGPT alkaline phosphatases bilirubin creatinine/s se rum electrolytes Albustix Clinistix and Coomb s test.

For statistical evaluation of the results presented in Table II the average systolic and diastolic blood pres sure levels measured at the end of the 10th and 1 th week of alprenolol treatment were compared to those *recorded after the corresponding periods of placebo* treatment Patient F pursued the placebo treatment for only 6 weeks (see Results) and the last blood pressure noted in the placebo period was taken to represent the placebo value in the statistical evaluation

Results are reported as means ± standard errors of the mean The statistical analysis was based on the *t* test (10)

RESULTS

The arterial blood pressure was lower during the alprenolol period than during the placebo period in ten of the eleven patients As shown in Table II there was after 10–12 weeks of alprenolol treat ment about the same average blood pressure re duction in supine as in sitting and standing posi tions the systolic pressure being decreased by about 20 mm Hg and the diastolic pressure by about 10 mm Hg

Figs 1 and 2 show the average supine and standing blood pressures recorded in each period

Table II *Average blood pressures recorded before and 10–12 weeks after treatment with alprenolol or placebo in the 11 patients*

	Initial control	After 10–12 *weeks on* alprenolol	After 10–12 *weeks on* placebo	Differences *alprenolol* placebo
Supine				
Systolic	175±4 7	153±4 7	176±2.9	−23±4 9 ($p<0\ 001$)
Diastolic	111±2.6	101±3 2	110±3 2	− 9±3 1 ($p<0\ 07$)
Standing				
Systolic	173±5 1	152±3 9	171±4 1	−19±4 9 ($p<0\ 01$)
Diastolic	121±2.3	108±2.1	121±2.8	−13±3 3 ($p<0\ 01$)
S tting				
Systolic	179±4 9	153±4 9	174±4 4	−21±6 1 ($p<0\ 01$)
Diastolic	115±3 0	103±2 5	114±3 2	−11±3 9 ($p<0\ 07$)

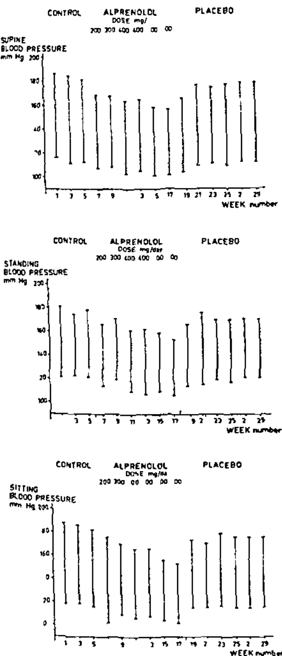


Fig 1 Average blood pressures recorded in the seven patients in whom the alprenolol period preceded the placebo period

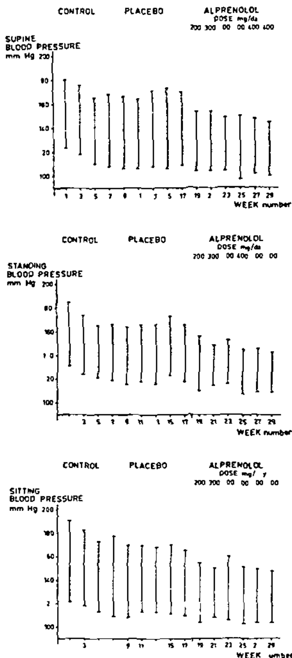


Fig 2 Average blood pressures recorded in the four patients in whom the placebo period preceded the alprenolol period

It can be seen that the blood pressure was reduced after two weeks treatment with 200 mg alprenolol daily and there was some further reduction during the following ten weeks of therapy When alprenolol was withdrawn the blood pressure returned to pre treatment control values within four weeks (Fig 1)

The individual time-effect curves during alprenolol treatment followed two different patterns In six patients (Fig 3) there was some decrease

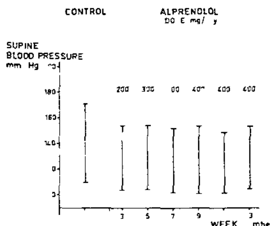


Fig 3 Average supine blood pressures before and during alprenolol therapy in six patients whose response to the drug was fully developed after two weeks treatment.

of the blood pressure after the first two weeks treatment with 200 mg alprenolol (mean reduction of supine systolic pressure 17 ± 5.1 mm Hg ($p < 0.05$) and of diastolic pressure 6 ± 3.3 mm Hg) and the blood pressure showed little further re during the following ten weeks with administration of higher doses In the remaining four patients who responded to the drug (Fig 4) the supine blood pressure was also somewhat reduced after the first two weeks of treatment but the blood pressure then continued to decrease throughout the following ten weeks of alprenolol therapy From the second to the last four week

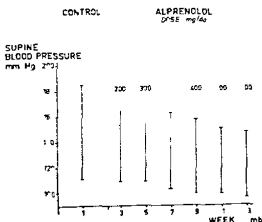


Fig 4 Average supine blood pressures before and during alprenolol therapy in four patients whose response to the drug developed gradually during the 12 weeks treatment.

period of alprenolol treatment the supine systolic blood pressure was in this group reduced by 12 ± 3.2 mm Hg ($p < 0.05$) and the diastolic pressure by 6 ± 1.6 mm Hg ($p < 0.05$) At the end of the 12 week alprenolol period the mean supine systolic and diastolic blood pressures of these patients were reduced by 36 ± 7.2 mm Hg ($p < 0.05$) and 23 ± 5.9 mm Hg ($p < 0.05$) respectively if the blood pressures recorded at the corresponding placebo period are taken as reference values

In the two groups of patients the standing and sitting blood pressures during alprenolol treatment followed on the whole the same differentiated time patterns as characterized the supine blood pressure The blood pressure variations during placebo treatment showed no striking differences between the two groups The patients with a gradual hypotensive response had on an average a somewhat higher control systolic blood pressure than the other group with a purely acute response but the difference (15 ± 8.5 mm Hg) was not statistically significant There was no obvious difference as regards age sex and severity of hypertensive disease between the two groups of patients

Two patients who showed a marked response to alprenolol deserve some comments Case F a 30 year-old woman was divorced and had several family problems She had typical symptoms of neurocirculatory asthenia i e palpitations sighing type of dyspnoea and uncharacteristic pain localized to the region of the apex of the heart This patient had a supine control blood pressure of about 200/125 Under alprenolol therapy the pressure showed a gradual fall to 140/100 at the end of the 12 week period (Fig 5) After withdrawal of alprenolol and introduction of placebo the blood pressure increased rapidly to pretreatment control levels The patient s treatment code was interrupted after six weeks placebo treatment and she was withdrawn from the rest of the study

Patient L was a 56 year-old man without previous signs or symptoms of coronary heart disease This patient responded promptly to two weeks therapy with 200 mg alprenolol daily his supine blood pressure being reduced from a control level of 180/105 mm Hg to 140/90 mm Hg The blood pressure remained at this level on treatment with 400 mg alprenolol except that the low value of 120/90 mm Hg was recorded after the

first two weeks on this dosage During these two weeks the patient complained of almost daily attacks of angina pectoris on exercise which however disappeared in the next period when the blood pressure apparently had stabilized at a slightly higher level

With the exception of the appearance of angina pectoris in patient L, no side-effects or complications were observed The laboratory investigations showed no significant changes in hepatic or renal function serum electrolytes or haematological status in any patient

DISCUSSION

The results with alprenolol in the present study correspond closely to those obtained in the parallel study by Furberg and Michaelson (14) The antihypertensive effect observed under alprenolol treatment is of the same order of magnitude as that reported for moderate doses of propranolol in several studies (13 20 31)

There are several reasons to assume that in the dosage most commonly used the antihypertensive effects of alprenolol and propranolol can be ascribed to their β receptor blocking action Haemodynamic studies carried out on hypertensive patients at rest indicate that the antihypertensive actions of alprenolol (3) and propranolol (3 13 27) are due to a reduction of cardiac output The anti hypertensive effects of propranolol and alprenolol during physical exercise have also been found to be due to a reduction of cardiac output (3 28) In alprenolol treated patients subjected to standardized exercise Furberg and Michaelsson (14) found a correlation between the decrease in the systolic blood pressure and the decrease in heart rate As could be expected for β blocking agents both alprenolol and propranolol have been found to produce greater absolute blood pressure reductions in situations of increased endogenous β sympathetic activity such as exercise (14 28) than they elicit under resting conditions On the other hand the agents do not produce such a marked hypotensive response to exercise as that which characterizes adrenergic neurone blocking agents (cf 22) Both alprenolol and propranolol have consistently been found to lower supine and standing blood pressures to about the same degree The lack of postural and exertional hypotension is to be expected since the homeostatic reflex control of the peripheral resistance and capacitance vessels is mediated via α adrenergic receptors in vascular

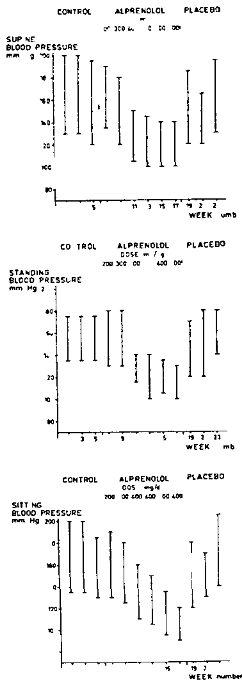


Fig 5 The effect of alprenolol treatment in a patient with hypertension and symptoms of neurocirculatory asthenia

smooth muscle (18) A single oral dose of 100 mg alprenolol elicits a marked cardiovascular β blockade approximately corresponding to that produced by 50 mg propranolol (32) Hitherto reported prolonged therapeutic studies with alprenolol and propranolol in hypertension indicate that there may be a similar 2 1 relationship between equipotent antihypertensive doses of the two drugs (13 14 20 31) In very high doses both alprenolol and propranolol have in addition to β blockade a direct depressant action on cardiovascular contractility (32) This mechanism might have been of contributory importance for the hypotensive effects reported by Prichard and Gillam (22) as considerably larger doses of propranolol up to 4 g daily were given to many of their patients

According to older concepts the hypertensive patient is haemodynamically characterized by an increased peripheral vascular resistance with normal or slightly reduced cardiac output (12) This seems still to be valid for most patients with severe hypertension and those with hypertension of long duration but more recent investigations indicate a different haemodynamic situation in many patients with mild or moderate hypertension who have an increased cardiac output with normal peripheral vascular resistance (1 2 9 15 17 25 30) There is some evidence that patients with high output hypertension develop high resistance hypertension as the hypertensive disease progresses (2 8) After intravenous administration of propranolol Bello et al (2) and Kuramoto et al (15) found a more marked reduction of cardiac output in patients with high output hypertension than in those with low output hypertension This finding indicates that the cardiac output in the former group of patients is at least to a substantial degree controlled by sympathetic tone Altogether the studies on the haemodynamic situation in hypertension discussed above make β receptor blockade an attractive therapeutic principle for patients in earlier stages of the hypertensive disease

In the present series the duration and the haemodynamic characteristics of the hypertension were unknown It is of interest however that of the two patients showing the most marked hypotensive response to alprenolol one was the only patient with clinical signs and symptoms of hyperkinetic circulation and the other was the only patient devoid of hypertensive eye ground changes Otherwise in this small series there was no obvious connection between the magnitude of the hypotensive effect on the one hand and the age of the patients and the severity of their hypertensive disease on the other

In the present study the patients could be divided into two groups in terms of the time curve for the hypotensive response to alprenolol In one group the full effect of the drug was developed after two weeks treatment while in the other group the effect continued to increase throughout the 12 week treatment period This finding gains more significance by the fact that the same differentiation could be made on re examination of the data obtained in the parallel study of Furberg and Michaelsson (14) The present data give no indication as to the cause of these different time effect curves From both practical and theoretical points of view it is of interest that in about one third of the patients in the present study alprenolol elicited a rather marked antihypertensive effect characterized by a gradual onset over several months Prichard and Gillam (22) have observed a similar gradual onset of the hypotensive response to propranolol These authors have suggested that as the cardiac β blocking effect of propranolol appears to be fully developed immediately after commencing the treatment the gradual hypotensive effect is due to a slow reconditioning of the baroceptors The studies of Frohlich et al (13) indicate that the antihypertensive effect observed after prolonged treatment with propranolol is still due to a reduced cardiac output

In the present study and in that of Furberg and Michaelsson (14) all but one patient tolerated a daily dose of 400 mg alprenolol without any complications or side-effects One patient in the present study reacted to this dose of alprenolol by a pronounced reduction of the blood pressure and complaints of exertional angina pectoris As this patient reached normotension on a daily dose of 200 mg alprenolol the appearance of angina pectoris after the higher dose could probably be regarded as a response to a supraoptimal therapeutic dose of the drug leading to a drastic reduction of the coronary perfusion pressure The reaction of this patient may indicate that when used in angina pectoris treatment an overdose of alprenolol may in certain cases produce a "para

dotical angina due to excessive hypotension as has also been described for nitroglycerine (24) The case reported above illustrates the importance of starting alprenolol therapy with lower dosage and of individualizing the maintenance dose Prichard and Gillam (22) have recently emphasized the importance of individualizing the dose of propranolol in antihypertensive treatment and report that a well tolerated normotension could be maintained on daily doses varying from 10 mg to 4000 mg In view of these data it seems well worth trying higher doses of alprenolol in patients who do not reach normotension on daily doses of 400 mg or below

Alprenolol and propranolol appear to reduce blood pressure to about the same degree but with different haemodynamic effect patterns Both after acute (2 29) and chronic (13) administration of propranolol to hypertensive patients the drug induced reduction of the cardiac output was as a rule accompanied by an *increase* of the peripheral vascular resistance This type of response to propranolol was also found by Bergman et al (3) who compared the acute haemodynamic effects of this compound and alprenolol in hypertensive patients in supine position Alprenolol reduced the blood pressure to the same extent as propranolol but *did not change* the peripheral vascular resistance The effects of propranolol were probably a consequence of β receptor blockade in the heart and peripheral blood vessels whereas the effects of alprenolol were presumably a result of β receptor blockade combined with a small β receptor stimulation in the heart and blood vessels (11)

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IMMUNOGLOBULINS IN SERUM AND JEJUNAL BIOPSIES IN NON TROPICAL SPRUE

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Abstract A study has been made of 12 patients with non tropical sprue Immunochemical determinations of serum IgG IgA and IgM were carried out prior to gluten free diet (9 patients) and during treatment (10 patients) Immunofluorescent microscopy of jejunal biopsies from the same patients was performed prior to (10 cases) and during treatment (6 cases) Fluorescent antisera specifically reacting with IgG IgA and IgM were applied and the cells containing these immunoglobulins were differentiated and quantitated accordingly

Serum IgM which was decreased in untreated patients was normalized during treatment. Serum levels of IgG and IgA were normal In both treated and untreated cases of sprue increased numbers of cells containing IgG and IgM were found The immunoglobulin levels of serum were not correlated to the area of cells containing the corresponding immunoglobulins

The human intestinal mucosa is known to contain a large number of plasma cells In immune histochemical studies it has been shown that the majority of these mucosal plasma cells contain IgA immunoglobulin (1 11) whereas cells containing IgM or IgG immunoglobulin are less numerous

Considering the very limited number of cells containing IgA immunoglobulin in lymph nodes bone marrow and spleen (8) it appears that a large proportion of the total body content of IgA containing cells is confined to the intestinal mucosa A correlation between the serum level of IgA and the number of intestinal plasma cells containing this immunoglobulin has been found in patients with marked hypo- or a gamma A globulinaemia (2 4 7) In sprue and Crohn s disease a similar parallelism between a moderately elevated serum IgA and intestinal cont nt of IgA cells has been found (4 10) However in the latter studies the determination of the intestinal content of IgA-cells was only semiquantitative In a recent report a deficiency in serum IgM was found in patients with untreated sprue During treatment with gluten free diet serum IgM was normalized (5)

The present study was undertaken to provide information about the correlation b tween the quantitatively determined intestinal amount of immunoglobulin containing cells and the serum immunoglobulin level in controls and in patients with non tropical sprue In addition the effect of gluten free diet on these parameters was studied

MATERIAL

Twelve patients aged 18 to 60 years with gluten induced enteropathy The diagnosis was based on clinical and biochemical evidence of malabsorption and subtotal or total atrophy of the proximal jejunal mucosa In all cases a clinical and biochemical improvement had taken place after a gluten free diet. Table I summarizes the findings in each patient Intestinal biopsy was performed in all cases to establish the diagnosis.

Immunofluorescent studies were carried out prior to treatment in ten cases and in six cases after 8–36 months on a gluten free diet

Serum immunoglobulins were determined before dietary treatment in nine cases and after dietary treatment in ten cases Blood samples and intestinal biopsy specimens were taken on the same day

METHODS

The concentration of IgG IgA and IgM in sera stored at −18 for 2 day –1 year was estimated by electrophoresis in antibody containing agarose (14) As refer

Table I

No	Sex	Age	Treatment	Duration of symptoms/ diet years	Faecal fat excretion	D xylose[b] absorption	Jejunal pathology	Immuno-fluorescent study performed	Serum immuno-globulin level determined
1	♂	61	No	4	48	2.1	Severe	No	No
			+	2	21	6 1	Severe	+	+
2	♀	58	No	2	75	1 1	Severe	+	+
			+	9/12				No	+
3	♂	17	No	7	23	1 5	Severe	+	+
			+	3/12				No	+
4	♀	50	No	8	5	8 0	Severe	No	No
			+	3	6	11 0	Moderate	+	+
5		44	No	30	33	5 1	Severe	+	+
			+	1	4	4 4	Severe	+	+
6	–	24	No	3	8	3 1	Severe	+	+
			+	9/12	2	7 9	Severe	+	+
7		26	No	2	15	3 2	Severe	+	+
			+	8/12	2			No	+
8		17	No	10	83	1 4	Severe	+	+
			+	9/12	18	Normal[c]	Severe	+	+
9	♀	27	No	1	70	3 3	Severe	+	No
			+	6/12				No	+
10	♀	54	No	30	30	4 2	Severe	+	+
11	♀	18	No	6	17	4 6	Moderate	+	+
			+	8 12	2	7 5	Slight	+	+
12		47	No	19	22	2 9	Severe	+	+

Grams per day from a 3-day stool collection Normal is less than 7 g.
Grams excretion in 4-hour urine collection after ingestion of 25 g Normal greater than 5 0 g.
Determined after blood values

in the quantitation Human Standard Serum Op no 166 (Behringwerke Marburg, Lahn Germany) was used The rabbit antibodies used were antihuman IgG (Dakopatts Ltd Copenhagen) antihuman IgA and anti human IgM (Behringwerke Marburg, Lahn Germany) The serum level of IgG IgA and IgM was determined in 44 healthy members of the medical staff (average age 33 years) and 55 blood donors (average age 39 years) The distribution of the immunoglobulin values showed a skrew distribution. In all the calculations therefore log values were used The 95% range for IgG was 7 2–15 1 g l (mean 10 4 g l) for IgA 0 74–3 06 g/l (mean 1.50 g l) and for IgM 0.23–1 33 g l (mean 0.56 g/l)

Biopsy specimen material

The jejunal biopsies were obtained with a peroral hydraulic multiple biopsy tube All specimens were taken 10–30 cm distal of Treitz ligament and were immediately frozen and stored at – 70 C

Immunofluorescent methods

The details of the procedure used for preparation of fluorescein-isothiocyanate conjugates as well as the technique of labelling with the fluorescent antisera, microscopy measurements and control of specificity have been fully described in a previous publication (12) and need only be briefly summarized here

Antisera against human IgA IgM and IgG were obtained from a commercial laboratory A crude globulin fraction was precipitated by 1 7 *M* ammoniumsulphate and was conjugated with fluorescein isothiocyanate at an alkaline pH By chromatography on a Sephadex G 25 column the free fluorescein isothiocyanate was removed from the antisera. Each conjugate was absorbed with guinea pig liver powder Tissue sections 4–6 μm thick were air-dried and fixed for 3 minutes in absolute methanol and preincubated with one drop of unconjugated anti IgA IgM or IgG sera (of the same batch as used for preparation of fluorescein conjugates) After washing in Coons buffer the sections were incubated with fluorescent anti IgA IgM or IgG

A Reichert Zetopan microscope equipped with a photo automaton was used for fluorescence microscopy Dark field technique was employed Photomicrographs were obtained under standard magnification with Anscochrome 24 DIN daylight film The photomicrographs were projected on a white sheet of paper The areas to be measured were drawn by hand cut out and weighed. As the immunoglobulin-containing cells were confined to the interstitial tissue they were quantitated by relating the fluorescent area to the total area of the interstitial tissue in a given visual field. Of each section used for quantitation six visual fields were examined and the mean of these determinations was used

In eight controls aged 21 to 60 years mean values (± S.D) of immunoglobulin-containing cells as % of

interstitial area were calculated. The following values were found IgA 14 4 ±6 9 IgM 5 8 ±3.5% I G 0 3% ±0.2%)

RESULTS

Serum Concentration of IgG IgA and IgM

The concentration of IgG IgA and IgM in serum before and during dietary treatment is shown in Fig 1

IgG

The mean serum IgG was 9 0 g/l (95% range 7 8–20 5 g/l) before treatment The mean serum IgG was 11 9 g/l (95% range 7 4–19 2 g/l) during treatment which is not significantly different from the normal mean ($p > 0.3$)

In seven patients the mean serum IgG before and during treatment changed from 8 4 to 10 7 g/l This alteration was not significant ($p > 0.05$)

IgA

The mean serum IgA was 1 56 g/l (95% range 0 27–7 28 g/l) before treatment and 1 62 g/l (95% range 0 52–6 41 g/l) during treatment which is not significantly different from normal ($p > 0.8$) In seven patients serum IgA was followed before and during treatment but the alteration in mean serum IgA from 1 39 g/l to 1 36 g/l is not significant ($p > 0.8$)

IgM

The mean serum IgM before treatment was 0 30 g/l (95% range 0 12–1 04 g/l) which was significantly lower than normal mean ($p < 0.001$) During treatment the mean IgM was 0 56 g/l (95% range 0 34–1 06 g/l) which is not different from the normal mean ($p > 0.9$) The mean serum IgM rose from 0 29 to 0 54 g/l in seven patients in whom it was determined before and during treatment This alteration is significant ($p < 0.005$)

Cells Containing IgG IgA and IgM in Jejunal Biopsies

IgG

The area of IgG cells as % of interstitial area was 9 9% (SD 7 3%) before treatment during treatment 5 3% (SD 2 3%) which in both cases are significantly higher than in controls ($p < 0.001$) In four patients the area of IgG cells was determined before and during treatment In these four patients a slight fall in IgG cells was seen

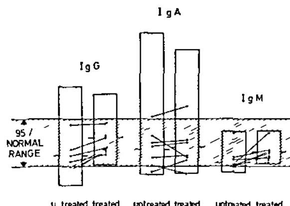


Fig 1 Concentration of IgG IgA and IgM in serum before and during dietary treatment in non tropical sprue 95% range is indicated

from 7 5% to 6 0% but this was not significant ($p > 0.3$)

IgA

The area of IgA cells in patients with untreated sprue was 18 0% (SD 9 7%) during gluten free diet 12 5% (SD 2 5%) which are not different from normal values ($p > 0.1$) In four patients the area of IgA cells was 15 3% before and 11 9% after start of diet This alteration is not significant ($p > 0.1$)

IgM

In untreated sprue the area of IgM cells was 12 1% (SD 2 4%) during treatment 9 9% (SD 3 6%) which are significantly higher than in controls ($p < 0.001$ and $p < 0.025$ respectively) In four patients the area of IgM cells was determined before and during treatment and a slight fall was seen from 12 7% to 10 3% but this is not significant ($p > 0.2$)

Correlation Between Immunoglobulin containing Cells in Jejunum and Serum Immunoglobulin Levels

Controls

In seven controls both serum values and jejunal area of plasma cells of the three immunoglobulins were determined The values are shown in Fig 2 No correlation between the serum level and the jejunal area of cells containing the same immunoglobulin could be found for any of the three immunoglobulins ($p > 0.1$)

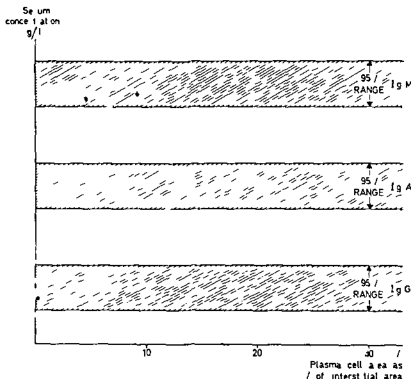


Fig 2 Correlation between the se rum level of IgG IgA and IgM and the area of jejunal cell con taining the same immunoglobulins in seven controls

Sprue patients

...ding serum values and jejunal interstitial ...ma cell areas for the three immunoglobulins ... shown in Fig 3 Values for both treated and untreated patients are included No correlation between the serum level and the jejunal plasma cell area could be found for any of the three immunoglobulins in untreated or treated patients ($p > 0.1$)

DISCUSSION

Serum IgM was significantly decreased in un treated sprue patients During dietary treatment

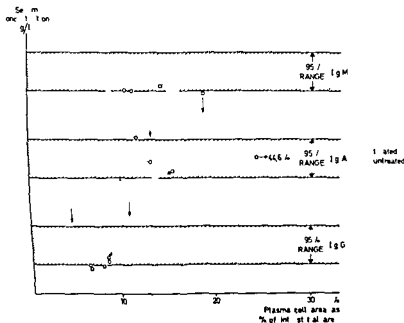


Fig 3 Correlation between the serum level of IgG IgA, and IgM and the area of jejunal cells con taining the same immunoglobulins in patients with untreated and treated sprue ▲ treated O un treated

serum IgM was normalized This is in agreement with the results published by Hobbs and Hepner (5) who found a similar low serum IgM which normalized during trea ment S rum IgA showed no alterations compared to normals either before or during treatment This was observed too by Hobbs and Hepner (5) but is in contrast to the report of Eidelman et al (3) and Poen et al (10) who found elevated values of serum IgA S^rum IgG was normal both in treated and untreated patients

The low serum IgM level might be due to gastrointestinal protein loss which is known to take place in untreated non tropical sprue (6 9 13) However a gastrointestinal loss cannot ex plain the discrepancy between the decreased serum IgM and the normal serum IgA and IgG and a selective loss of the high mole~ular IgM is un likely A d creased IgM synthesis seems not very likely as the immunofluorescence micro copy showed a significantly elevated number of IgM containing cells in the jejunal mucosa

Further elucidation of these problems must await protein turnover studi s

The results of the immunofluorescent studi^es in non tropical sprue have been published in part elsewhere (12) As in normals the IgA cells are most numerous in th jejunal mucosa but a significant increase in IgM and IgG containing cells was found in both groups of sprue pati nts and the total number of immunoglobulin contain ing cells was increased In an immunofluorescence study of intestinal biopsies from sprue patients Rubin et al (11) found a similar increas^ of the immunoglobulin containing cells but noticed a larger predominance of IgA cells than found in this study This difference might be due to the composition of the case material as the jejunal pathology in the pres nt study appeared to be more pronounced than in the material of Rubin et al

In patient number 3 who had normal serum concentration of immunoglobulins an extreme elevation of the total number of immunoglobulin containing cells in the jejunal mucosa was du to an increase in the IgG and IgA cells The pa ient was infantile It is under investigation wh^ther the infantilism is primary or secondary

No correlation was found between corr^sp^nd ing valu s of the serum concent ation and the area of immunoglobulin-containing cells ir je junum for any of the immunoglobulins studied As a consid rable part of the IgA containing cells of the body are probably found in the intestinal wall a positive correlation could be expected as far as IgA is concerned

In sprue patients in whom both elevated serum IgA and jejunal area of IgA cells have been de scribed (3 10) the proper condition for demon strating this corre^lation should b present In fact we did not find any alterations of IgA in serum and only a slight but insignificant increase of IgA cells in jejunum and with th^se minimal changes no correlation could be demonstrated

ACKNOWLEDGEMENTS

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Addendı m After completion of the study it appeared that the anti IgA preparation employed had also some specific activity against secretory p ece since colostrum had been used for immunisat on Thus a new anti I_A preparation was procured This preparation was tested in serial d lution against normal human s rum l ght chains myeloma and Waldenstrom sera and a_ainst colostrum It proved to possess specific reactivity only a_ainst serum IgA Seven of the biops es (3 controls, 4 pat ents with non tropical sprue) included in this in vestigation were re-examined with the new and the old ant IgA preparation

Quantitation stud es showed that there was no differ ence in the number of interstitial cells giving specific fluorescence with the two antisera The faint stain ng of the epithel al cells induced by the anti IgA with activity aga nst secretory piece was absent with the new anti IgA preparation and consequently this stain ng cannot be considered unspecif c Stud es with specific ant sera aga nst secretory piece and serum IgA are now be ng undertaken

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STUDIES OF PITUITARY ADRENOCORTICAL FUNCTION IN ULCERATIVE COLITIS

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Abstract The pituitary adrenal function has been evaluated in 41 patients with well defined ulcerative colitis No dysfunction was shown In three cases a false negative response in urinary 17 KGS was found during the metyrapone test.

The variable course of ulcerative colitis and the beneficial effect of glucocorticoids in some cases may suggest a relative insufficiency of the pituitary adrenal system as a causal factor in exacerbations of the disease This could parallel findings in bronchial asthma in which Robson and Kilborn (12) found a diminished response of the adrenal cortex to exogenous corticotrophin in 21 out of 34 patients with continuous asthma

The purpose of the present study was to evaluate the pituitary adrenal function in a group of patients with well defined ulcerative colitis It was done by means of the plasma concentration of 11 hydroxy-corticosteroids (11 OHCS) and the urinary excretion of 17 ketogenic steroids (17 KGS) spontaneously and during the administration of corticotrophin and metyrapone

MATERIAL AND METHODS

The material comprised 41 patients with ulcerative colitis consecutively admitted to the Gastroenterological Department B The age of the patients varied from 16 to 75 years The distribution with respect to age and sex was not different from that found in a larger group of patients with ulcerative colitis (4) The diagnosis of ulcerative colitis was based upon the fulfilment of at least two of the following four sets of diagnostic criteria

1 A case history including bloody diarrhoea and/or passage of blood between the proper bowel movements The presence of diarrhoea and/or rectal tenesmus is not necessary

2 Sigmoidoscopical findings (at least two of the following findings must be present) (*a*) granulated mucosa (*b*) increased friability (*c*) haemorrhagic and/or purulent contents in bowel lumen (*d*) ulcerations punctate or larger (*e*) pseudopolyps

3 Cytological and/or bioptical signs of inflammation Cytology presence of inflammatory leucocytes (presence of eosinophils not necessary) Biopsy presence of inflammatory leucocytes in the mucosal and submucosal layers.

4 Radiological finding of (*a*) spicula (*b*) small or large ulcers (*c*) pseudopolyps (In patients presenting colonic diverticula three instead of two major diagnostic criteria headings 1–4 to be fulfilled for the diagnosis of ulcerative colitis)

None of the patients had been treated with glucocorticoids within one year prior to the study and 39 of the patients had never been treated with glucocorticoids

The excretion of 17 KGS was determined in 24-hour urinary specimens by the method of Jørgensen (10) at the Hormone Department of Statens Seruminstitut Copenhagen

The plasma concentration of 11-OHCS was determined using the fluorimetric technique of DeMoor et al (8) as described by Nielsen and Asfeldt (11) For determination of cortisol and 11-deoxycortisol a double isotope technique was used (3 6) The latter two analyses were carried out by Medicinsk Laboratorium Copenhagen.

All the patients were admitted to hospital during the study None of them were given sedatives or narcotics After a two-day control period the ACTH test was carried out If the concentration of 11-OHCS in plasma was initially normal and had doubled or more 4 hours after the i m administration of 30 I U corticotrophin (Acton prolongatum®) the test was considered positive (1) If this was not the case the administration of corticotrophin was continued 6-hourly for 3 days A doubling of the daily excretion of 17 KGS was considered an adequate response from the adrenal cortex (9)

After a 3-day interval the metyrapone test was carried out starting with a new 24 hour urine collection Then metyrapone was given 4 hourly in 500 mg doses for 24 hours beginning at noon A blood sample for the specific determination of cortisol and 11 deoxycortisol was drawn

Acta med scand Vol 186 pp 469–473 1969

THE SERUM IMMUNOGLOBULIN AND β 1 C/β 1 A GLOBULIN LEVELS IN RHEUMATOID ARTHRITIS

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Abstract The immunoglobulins IgG IgA and IgM and the β 1 A globulin have been determined by the single radiation immunodiffusion technique in serum samples from 55 RA patients and 48 healthy controls The average total Ig level and the IgG level were higher in RA sera The IgA and IgM levels were also somewhat higher but the d fference was not statistically significant if the age group was taken into consideration The β 1 A globulin content was similar in the RA sera and the controls This globulin was positively correlated to the total Ig and to the IgG level in the RA series and inversely to the IgM level in the normal series The IgG level rose w th increasing age both in the RA series and in the whole series In the normal series the IgM level diminished with increasing age

The auto-immune nature of rheumatoid arthritis (RA) has not been unequivocally established even if it has been suspected for a long time (1 4 12) The rheumatoid factor (RF) is an immunoglobulin of the IgM class reacting with human or animal IgG (1 11 12) For diagnostic purposes RF is demonstrated in the serum by the Waaler Rose test or similar procedures but the factor has also been found in the tissues of patients suffering from RA (1 8 9 15) RA might thus b characterized as a disease accompanied by noteworthy immunological phenomena even if the exact pathogenetic role of RF is still unknown These phenomena are mainly humoral and changes are to be expected in the levels of circulating immunoglobulins (Ig) and complement (C′)

In active RA the total Ig level is usually high (2 3 21 23) and raised values of IgG were found by Muller and Muller von Voigt (16) in a small material Mackievicz and Fenrych (13) found elevated IgM values in most cases of RA Svartz (19) on the other hand found very high values in some cases but most of her RA patients had normal amounts of IgM in their serum By the application of a titration method on agar plates we (25) have previously found larger amounts of IgM in the serum of RA patients than in that of other hospital patients and of healthy blood donors Veys and Claessens (24) found normal IgM levels in RA according to them the age factor is very important and Ig levels should be compared only in corresponding age groups Within the age groups the average levels of IgG IgM and IgA in serum samples from patients with RA did not differ significantly from normal sera although in RF positive sera the levels of all three Ig classes tended to be raised

The total C activity of serum from patients with RA has been studied by several groups of research workers and values which are normal to somewhat elevated have been recorded in most cases (3 7 19 26) However Gross et al (5) found low values in 10 of 24 cases and Boni (3) established that the values are low in malignant forms of the disease Hanauer and Christian (6) determined both the total C and C 1 and found normal or elevated values in four cases but low values in a patient seriously ill with arteritis Pet kin and Zvaifler (18) noted diminished C activity in the synovial fluid of RA patients and according to Hedberg (7) it is negatively correlated to the serum RF titre

Quantitatively β 1 C globulin is the most im portant fraction of C (20) It belongs to C 3 and is in vitro converted to β 1 A globulin which can be determined by precipitation in agar gel (10 17 19) A close correlation has been found be tween this globulin and the total C′ activity (17)

Table I *The average levels of immunoglobulins and β 1 A globulin in RA sera and in normal sera*

Type of protein	Series	No of observations	Mean (mg/100 ml)	S D	p-value
Total Ig	RA	51	2172	815	<0 0005
	Normal	46	1654	402	
IgG	RA	52	1571	671	<0 0005
	Normal	48	1188	337	
IgA	RA	52	383	242	<0 05
	Normal	47	308	156	
IgM	RA	51	198	106	<0 05
	Normal	47	164	71	
β-1-A	RA	55	98	24	>0 1
	Normal	48	104	37	

Muller and Muller von Voigt (16) have applied a modified Oudin technique for determination of the amount of β 1 A globulin in sera from RA patients Although the average value was higher than in normal sera the difference was not statistically significant.

The present investigation was aimed not only at a comparison of the levels of IgG IgA IgM and β 1 A globulin in the serum of RA patients with the serum levels in normal controls but [illegible]larly at a study of the correlation of these [illegible]r globulins to each other and to certain other [illegible] such as age duration of the disease sedimentation rate RF titre antinuclear antibodies and the degree of anaemia

MATERIAL AND METHODS

Serum samples were obtained from 55 patients suffering from rheumatoid arthritis and from 48 healthy blood donors. All the patients had typical RA by the criteria of the American Rheumatic Association. About half of them were hospital patients the others attended the outpatients department. The blood donors were selected to represent age groups and a sex ratio similar to those of the RA patients. The patients and the healthy subjects were divided into two age groups, one less than 55 years and one 55 years or more This age limit was chosen simply because by this means both the patients and the normal controls were divided into two categories of almost equal size

The serum samples were kept overnight at room temperature and then for 3 days at +4-6 C to complete the in vitro conversion of β 1-C to β 1 A globulin (17) and were later stored for varying times at −20 C The levels of IgG IgA, IgM and β 1 A globulin were assayed by the single radial imunodiffusion method of Mancini et al. (14) with commercially available Partigen® plates from Behringwerke Inc., and standard sera from the same firm The values given by the producer were not checked, since the main purpose of the study was comparison of the Ig and C levels in RA sera with normal controls, and not the estblishment of normal values in a Finnish population Four of the 12 wells on each plate were filled with 2 μl of different dilutions of standard sera, and the others with dilutions of the unknown sera The plates were first kept overnight at 37 C, to preclude errors arising from cryoprecipitation, and then at room temperature The diameters of the precipitation rings were measured after 2 days with a micrometer magnifier using dark field illumination In most cases the rings were single *circular and distinct and the standard curves were linear* Results which did not meet these criteria were discarded.

The arithmetical means were calculated, and the significance evaluated by the *t* test. Computer analysis was used for this purpose and for the calculation of correlation factors

The immunofluorescent tests for detection of antinuclear antibodies were carried out by Dr O Wager and Dr J Rasanen of the municipal immunology laboratory at the Aurora Hospital, Helsinki cryostat sections of mouse liver were used as substrate

RESULTS

Immunoglobulin and β 1 A globulin levels

It was found that both the total Ig and the IgG levels were significantly higher in the RA series than in normal sera (Table I and Fig 1) Moreover the IgA and IgM levels seemed to be somewhat higher in the RA series than in the control series although the difference was not statistically significant in either age group when the two age categories were considered separately (see below under Discussion) No significant difference was noted between the average serum levels of β 1 A globulin

Age and sex

In the normal series no Ig seemed to be sex dependent whereas in the RA series the IgA level was significantly higher in males than in females ($p<0\ 02$) The total Ig, and the IgG levels (Fig 1) rose with increasing age in the case of the normal subjects ($p<0\ 01$) The IgM level on the other hand was significantly lower in the higher age group The difference was insignificant when the means of the two main age categories were compared (Fig. 2) but computer analysis revealed a reciprocal correlation between age and serum IgM concentration on the 98 per cent level No significant correlations between age and Ig values were found in the RA series

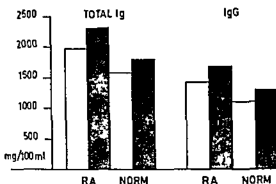


Fig 1 The average total Ig levels and IgG levels in the two age categories. White columns < 55 years black columns ≥ 55 years

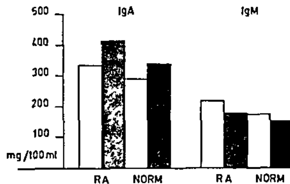


Fig 2 The average IgA and IgM levels in the two age categories White columns < 55 years black columns ≥ 55 years

Correlations between the immunoglobulins and the β 1 A globulin

Among the normal subjects the β 1 A globulin level seemed to be inversely correlated to the serum level of IgM ($p < 0.05$) In the normal series a positive correlation was found between the serum levels of IgA and total Ig ($p < 0.001$) In the RA series a positive correlation existed between the IgG and IgA levels ($p < 0.001$) An almost significant positive correlation was also noted between both the β 1 A globulin and total Ig levels ($p < 0.05$) and between the β 1 A globulin and IgG levels ($p < 0.05$) No such correlations were found in the normal series

Other correlations (Table II)

In the RA series the ESR value was positively correlated to the total Ig The ESR value also seemed to be positively correlated to the IgG and IgA levels and was not very surprisingly reciprocally correlated to the haemoglobin level

The degree of Latex positivity was correlated to the total level of Ig and also seemed positively correlated to the level of IgG A correlation could be demonstrated between the degree of positivity of the Waaler Rose reaction and the amount of polyvalent anti nuclear antibody in the patient serum The amount of IgM type antinuclear antibody in patient serum was positively correlated to the serum level of IgA A rise seemed to occur in the serum level of IgA with increasing duration of illness while no such correlation was observable in the case of the other Ig s

DISCUSSION

Various immunological phenomena accompany the disease process in rheumatoid arthritis although their pathogenetic significance is unknown An effect is to be expected on the circulating levels of the immunoglobulins if the processes are of humoral type and on complement if they require complement factors in vivo However the

Table II *Correlation survey RA series*

	Total Ig	IgG	IgA	IgM	β–1–A
Duration of disease	—	—	$p < 0.05$	—	—
Hb value	—	—	—	—	—
ESR	$p < 0.01$	$p < 0.05$	$p < 0.05$	—	—
Latex titre	$p < 0.02$	$p < 0.05$	—	—	—
Waaler Rose titre	—	—	$p < 0.05$	—	—
Anti nuclear antibody					
polyvalent	—	—	—	—	—
IgG type	—	—	—	—	—
IgM type	—	—	$p < 0.01$	—	—

— No significant correlation

influence on the levels of circulating globulins cannot be predicted, as an increased consumption is probably followed by an increased production and the combined result may be high normal or low levels

The higher total Ig levels and the IgG levels found in sera from RA patients probably result from imunological stimulation of antibody production The increase was more pronounced in severe cases with high ESR and low haemoglobin values The IgA and IgM levels were also increased to some extent in RA patients but only on the 95% level statistically The correlation between the IgM antinuclear antibody titre and IgA level is surprising, and theoretically difficult of explanation Even if the correlation was observable on the 99% level it must be stressed that only 13 sera contained such antibodies and the finding may consequently be incidental The increase in the IgM levels in RA patients was less pronounced than in our previous study in which different techniques were applied Within the age groups (see below) the difference was not statistically significant It is well known that in most cases the rheumatoid factor is an IgM globulin and RF vits in vivo would probably suppress the circulating level even where the production increased However no correlation either positive or negative was found between the Latex or Waaler Rose titre and the IgM level This observation too is explicable by a proportional effect on the production and on the consumption of IgM

The influence of age did not prove to be as important as had been reported by Veys and Claessens (24) In the combined series of RA patients and healthy blood donors only the IgG level rose somewhat with increasing age ($p < 0.05$) However we agree with Veys and Claessens that the age factor must be taken into consideration. In the present series the higher IgA and IgM values found in RA patients did not differ significantly from the normals within the age groups This is somewhat surprising, since the average age of the RA patients was only five years higher than that of the normal controls and the IgA and IgM levels were not found to be significantly correlated to the age in the whole series of RA patients and healthy people In both age categories the average IgA and IgM levels were higher in RA sera (Fig 2) but by reason of the smaller numbers of sera tested in the divided material and the high standard deviations (Table I) the differences were no longer statistically significant.

The β 1-C globulin, a moiety of C 3 was selected for study as representing serum complement factors since serum contains higher amounts of this protein than of other complement fractions, and since it is easily determinable by the radial immunodiffusion technique The average amount in the whole series (RA and controls) was found to be 100 5 mg per 100 ml It varied less from case to case than did the immunoglobulins the standard deviation was 30 9 A slight increase was noted to correlate with increasing total Ig, and with IgG levels ($p < 0.05$) in RA sera Thus, if there is an increased IgG antibody response the β 1-C globulin production also seems to increase

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PLASMA VOLUME COLLOID OSMOTIC PRESSURE AND GAMMA GLOBULIN IN MULTIPLE MYELOMA

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Abstract Serum protein concentration colloid-osmotic pressure and plasma volume have been determined in fourteen patients suffering from multiple myeloma A positive correlation was found between gamma globulin and plasma volume ($r = 0.74$ $p < 0.0005$) The colloid osmotic pressure was found to be relatively high eight out of 20 recordings ranging above 46 cm of water It is claimed that the relationship between gamma globulin and plasma volume depends on the plasma-expanding effect of gamma globulin Parallels are drawn to experimental hyperglobulinaemia

Animal experiments have shown that in hyperimmunized rabbits an increase in gamma globulin is accompanied by an increase in plasma volume (3 4) In spite of pronounced variations in the gamma globulin concentrations the colloid-osmotic pressure is normal (6 9) Most likely this phenomenon is due to the fact that in high concentrations the gamma globulin acts as a plasma expander" because of its colloid-osmotic pressure In multiple myeloma gamma globulin concentrations are found which are similar to those in the above mentioned experiments The object of the present study was to clarify inter alia whether a relationship exists between plasma volume and gamma globulin also in such cases Furthermore the colloid-osmotic pressure was studied in order to find out whether it remained normal in spite of the appreciable production of protein in multiple myeloma Finally an investigation was made of the relationship between actually measured colloid-osmotic pressure and colloid-osmotic pressure calculated on the basis of the plasma protein concentration in order to see whether an aggregation or a dissociation of the protein molecules takes place A preliminary report on the study has already been published (6)

MATERIAL AND METHODS

Fourteen patients suffering from multiple myeloma with IgA and IgG myeloma protein were examined in 1967 and 1968 (Most of the patients were examined at Bispebjerg Hospital Department B Some of the patients have been examined during their stay at the University Hospital of Copenhagen Department A the Copenhagen County Hospital in Gentofte Department C and the Finsen Institute Copenhagen Medical Department.) The sex and age of the patients are shown in Table I All the patients were examined for the purpose of revealing conditions if any which might influence the plasma volume In particular examinations aimed at revealing cardiac failure and gastro intestinal disorders were carried out

The blood samples were drawn in the morning with the patient lying down Serum gamma globulin was determined by paper electrophoresis adopting the method of Laurell et al (10) No correction was used for the varying stainability of gamma globulin Gamma globulin was taken to represent myeloma protein plus normal gamma globulin The total protein determination was made using a biuret method Plasma volume was determined as the 10 min distribution volume of intravenously injected ^{131}I labelled serum albumin or Evans Blue

Colloid-osmotic pressure (c o p) in serum was determined by the Tybjaerg Hansen method (8) (The determinations were carried out at the Department of Clinical Physiology Bispebjerg Hospital) Colloid-osmotic pressure was calculated from the equation

$$c\,o\,p = 1\,33 \times (4\,20A + 0\,33A^2 + 2\,35G)$$

where A is the serum albumin and G the serum gamma globulin concentration (6) The coefficient of variation is 10

RESULTS

The results appear in Table I presenting one measurement from each of the 14 patients It will be seen that the plasma volume increases with increasing levels of gamma globulin Table II shows all measurements from the five patients in

Table I *Serum gamma globulin colloid osmotic pressure and plasma ... in fourteen cases of multiple myeloma*

No	Sex	Age	Type of myeloma protein	Weight (kg)	Haemoglobin (g/100 ml)	Serum albumin (g/100 ml)	Serum gamma globulin incl paraprotein (g/100 ml)	Colloid osmotic pressure (calculated cm of water)	Colloid osmotic pressure (observed cm of water)	Plasma volume (ml)	Plasma volume (ml/kg)
1*	♀	58	IgA	71 5	7 4	3 28	1 72	?8 5	49 1	3390	47 4
2	♂	67	IgG	72 5	9 0	3 37	2 78	32 6	37 4	3270	45 2
3	♂	70	IgA	63 0	10 0	4 45	2 82	4? 5	42 1	2990	47 4
4	♀	63	IgG	95 0	8 2	3 71	3 06	36 4	37 0	3610	38 0
5	♀	56	IgG	71 6	11 9	3 96	3 61	40 3	45 0	2490	34 8
6	♀	62	IgG	74 0	9 4	4 15	1 95	43 1	44 7	2530	34 2
7	♂	65	IgA	68 0	10 8	3 51	4 16	38 1	43 4	3?00	47 0
8	♀	70	IgG	55 2	9 5	2 89	5 46	37 0	42 9	?860	51 8
9	♂	37	IgG	61 0	10 7	3 92	6 12	46 9	44 4	3800	62 3
10	♂	73	IgA	52 0	9 8	2 77	6 40	38 9	45 9	3630	69 8
11	♀	70	IgG	60 6	10 8	3 07	7 ?0	44 0	41 8	2870	47 4
12	♀	71	IgA	73 0	5 9	1 78	9 13	40 4	44 3	4050	55 4
13	♂	64	IgA	69 0	6 3	3 12	9 15	50 5	51 8	5530	80 2
14	♀	81	IgG	53 0	5 8	2 95	11 06	55 0	53 8	36 0	68 3

Uraemia

Table II *Serum gamma globulin colloid osmotic pressure and plasma volume studied two to four times in five cases of multiple myeloma*

No	Date	Weight (kg)	Haemoglobin (g/100 ml)	Serum albumin (g/100 ml)	Serum gamma globulin incl paraprotein (g/100 ml)	Colloid osmotic pressure (calculated cm of water)	Colloid osmotic pressure (observed cm of water)	Plasma volume (ml)	Plasma volume (ml/kg)
4	30 8 67	104 5	5 9	3 24	2 13	29 4	28 1	5310	58 1
—	13 9 67[a]	95 0	8 2	3 71	3 06	36 4	37 0	3610	38 0
6	11 9 67	74 0	9 4	4 15	3 95	43 1	44 7	2530	34.2
—	21 9 67	74 0	9 8	4 15	3 95	43 1	46 7	2340	31 6
7	9 5 68	68 0	10 8	3 51	4 16	38 1	43 4	3.00	47 0
—	6 6 68	66 0	6 8	2 36	6 50	36 0	44 8	4210	63 7
9	6 4 68	61 0	10 7	3 92	6 12	46 9	44 4	3800	62 3
—	1 7 68	65 0	13 5	4 41	4 59	47 7	45 3	3330	51 2
12	8 4 67	73 0	5 9	1 78	9 13	40 4	44 3	4050	55 4
—	30 5 67	70 0	6 9	2 32	6 10	34 4	34 4	31 0	44 5
—	8 6 67	68 9	7 1	. 46	2 77	25 1	25 8	690	39 0
—	6 7 67	67 5	10 6	3 61	1 85	31 7	26 5	670	39 6

Incompensated heart disease   * Compensated h rt disease   M lph l n * tre tm nt Blood tran f ions (500 ml) gi en on July 6 7 and 8

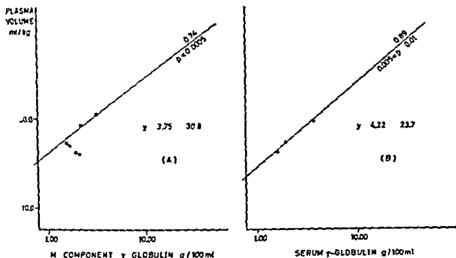


Fig 1 (A) The relation between plasma volume and gamma globulin (normal gamma globulin + paraprotein) in multiple myeloma (0 analyses in 14 patients) (B) The relation between plasma volume and gamma globulin in hyperimmunized rabbits

whom two or more measurements were taken during the course of the disease In patients 6 7 9 and 12 the plasma volume tends to vary with changes in the concentration of gamma globulin This is particularly evident in patient 12 on whom several tests were made during the course of the disease Patient 4 is an exception In this case the highest plasma volume was found at a time when the patient suffered from severe cardiac failure with oedema whereas the lowest plasma volume was measured when after appropriate treatment, the failure had been alleviated The corresponding gamma globulin values were 2 13 and 3 06 g per 100 ml respectively and hence they varied in versely with the plasma volume All interdependent plasma volumes and gamma globulin values are shown graphically in Fig 1(A) with the exception of those measured in patient 4 for whom only ne value recorded while the heart disease was ell compensated is given It will be seen that a istinct correlation exists between the plasma vol ime and the concentration of gamma globulin he correlation coefficient is 0 74 ($p < 0\,0005$) he equation for the regression line is $y = 3\,75x + 30\,8$

The correlations between the haemoglobin con entration and the plasma volume and between he albumin concentration and the plasma volume were also calculated In both cases sligthly nega ive correlations were found (haemoglobin and plasma volume $r = -0\,38$ $p < 0\,05$ albumin and plasma volume $r = -0\,38$ $p < 0\,05$)

In two patients (no 7 (6 6 68) and no 13 (19 7 68)) the central venous pressure was measured and found to be normal, 10 and 9 mm Hg, respectively at a plasma volume of 63 7 and 80 2 ml/kg

The measured colloid-osmotic pressure was found to range between 25 8 and 53 8 cm of water The calculated colloid-osmotic pressure varied from 25 1 to 55 0 cm of water

DISCUSSION

The study revealed a positive correlation between gamma globulin concentration and plasma volume in patients suffering from multiple myeloma This positive correlation can be observed also in the individual patients in connection with changes in the gamma globulin concentration The patient (no 4) who suffered from cardiac failure is an exception as the plasma volume under these con ditions increases with cardiac failure and decreases wh n the failure is alleviated Presumably this phenomenon explains why it has not been possible to demonstrate earlier any correlation in materials published by Gabuzda (7) and Andersen (1) How ever when the case reports includ*d in Ander sen s material are analysed and one patient with cardiac disease is excluded, there seems to be a

correlation (6) The literature relating to this subject is however scarce Birke (2) mentions briefly that there is a highly significant increase of plasma volume in hypergammaglobulin cases but apart from this finding no other mention appears to have been made As stated above animal experiments have shown that in hyperimmunized rabbits there is a positive correlation between gamma globulin and plasma volume Fig 1(B) presents results reported previously (4) obtained in 13 hyperimmunized rabbits The correlation coefficient is 0 88 ($p < 0\ 01$) and the equation for the regression line is $y = 4\ 22x + 23\ 7$ The difference in slope between the regression line for patients with myeloma and immunized rabbits is not significant Hence these experimental results seem to be parallel to the conditions found in multiple myeloma

There is a possibility that the production of gamma globulin in multiple myeloma might reach levels of such a height that an overloading would occur with a high central venous pressure similar to the conditions seen in connection with excessive transfusions of blood and plasma How normal central venous pressure was ob ed in two patients with a very high plasma ıme

In the present material the colloid-osmotic pressure was found to be surprisingly high eight out of 20 readings being definitely above 46 cm of water which is the upper normal limit for the method employed A comparison of measured and calculated colloid-osmotic pressure shows that in one case (patient 12 26 7 67) the measured pressure was sligthly below the calculated pressure and in another (patient 1) the measured pressure was considerably higher than the calculated pressure

It is thought that the difference between measured and calculated colloid-osmotic pressure may be explained by aggregation of protein molecules The patient in whom a lower measured than calculated pressure was found did not present any clinical signs of aggregation of protein molecules associated with the hyperviscosity syndrome (11) The size of the molecules was not determined by ultracentrifugation The other patient in whom the measured pressure was considerably higher than the calculated, suffered from severe uraemia presenting a serum creatinine level of 12 mg per 100 ml Our continued studies will show whether uraemia exerts any influence on the determination of the colloid-osmotic pressure with the method employed

ACKNOWLEDGEMENT

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THE CONTROL OF PLASMINOGEN AND PLASMIN DURING THROMBOLYTIC THERAPY

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Abstract Plasminogen levels during thrombolytic therapy have been recorded utilizing a previously standardized method The plasminogen is determined as proactivator in a rapid clot lysis system The method seems to be reliable and may be used as an alternative to the casein olyti assays

Plasminogen and plasmin are important factors in the modern principles of streptokinase treatment A marked fall in plasminogen and subsequent rapid elimination of plasmin from circulation is desired, in order to avoid proteolysis of plasma components (7 11 22) The mechanism in uro kinase treatment may possibly be somewhat dif ferent but this is not finally established (8)

In the present work a previously described method for determination of human plasminogen probably also including plasmin has been used (2 3) The assay is based upon a clot lysis system and has proved valuable as a rapid evaluation of the plasminogen (and plasmin) level during throm bolytic therapy with streptokinase and urokinase

MATERIAL

Anti coagulant Sodium citrate dihydrate 3 13% For clot l ng studies the citra e solution contained 5 mg of epsilon amino-caproic acid and 2500 units of Trasylol per ml in order to avoid fibrinogenolysis in vitro These con centrations of inh'bitors did not nfluence the clottin systems

Buff r A modified veronal buffer (pH 7 35 and ionic strength 0 15) (0)

Streptokinase (Streptase) from Hoechst, Behringwe ke AG Marburg/Lahn, Germany thrombin (Topostasin) from Roche Basel, Switzerland (expressed in N I H units) and urokinase from Roche Basel Switzerland (expressed in C T A units)

METHODS

Collection of blood Nine parts of blood were mixed with one part of anticoagulant at 4 C Plasma was prepared by centrifuging at 4 C for 30 min at ›500 rpm (1400 *g*) and stored in aliquots at −70 C

Fibrinogen was determined as fibrin after coagulation with thrombin by the method of Jacobsson (13) with the modification of Blomback and Blomback (5) and Godal (9) For the collection of blood anticoagulant with addi tion of f brinolytic inhibitors was used (see Material)

Plasm nogen determinat on

1 Plasminogen was determined as proactivator (P.A) by the standardized method of Blix (3) 0 10 ml citrated bovine plasma 0 05 ml streptokinase (8000 U/ml buffer) 0 05 ml citrated human test plasma (diluted 1 10 in buffer) 0 10 ml thrombin (30 U ml buffer) If desirable all volumes may be doubled

All reagents were kept on ice water The bovine plasma was incubated for 15 sec at 37 C in glass test tubes (10 × 70 mm) and the other materials were added at 15 sec intervals. The time from addition of thrombin to complete lysis of the clot was recorded. Soon after the clot had formed, small air bubbles could be observed in the clot, as described by Blomback et al (4) and Lassen (17) A few seconds before the clot had been lysed, all the air bubbles rose to the surface When they reached the surface the clot was completely lysed and this point was easy to record The test tubes should not be tilted but carefully raised at intervals for inspection of the air bubbles All tests were performed in duplicate

The per cent of plasminogen (proactivator) is read on a reference curve obtained from dilution series of pooled normal plasma (Fig 1) Due to the slope of the curve plasmas with normal or higher concentrations of plasmin ogen m ght better be tested in dilution 1 20 rather than 1 10 and the per cent value multipl ed by 2

The most conven ent lys s time at the 100% level is between 120 and 00 ec Some bovine plasmas will gi e too long lysis times but this can be corrected by in creasin the concentration of thrombin (?)

2. Plasminogen was also determined by a caseinolytic method after acidification of plasma with HCl and sub-

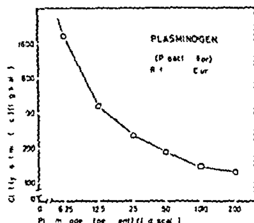


Fig 1 The reference curve for calculation of the plasminogen (proactivator) concentration in human plasma. The abscissa gives the values in per cent of normal obtained by dilution series of pooled, normal human plasma in buffer (100% is plasma tested in dilution 1 10)

sequent neutralization with NaOH A 1% casein solution was used as substrate and the plasminogen converted to plasmin by means of urokinase The incubation period was 60 min

Thrombin time was recorded in this system 0.2 ml citrated plasma 0.1 ml thrombin (6 U/ml saline) Normal clotting time 13–15 sec

For the collection of blood anticoagulant with addition fibrinolytic inhibitors was used (see Material)

ADMINISTRATION OF THE THROMBOLYTIC THERAPY

Administration of streptokinase

Three patients are presented in this report The initial dose was 250 000 units in 20 ml saline during 15 min.

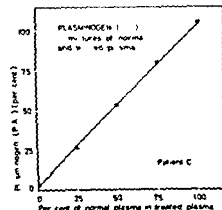


Fig 2 Mixtures of plasmas from a patient before and during treatment, as tested in the present plasminogen system (ordinate) The plasminogen in the treated plasma was found to be 3.5 % when controlled by a caseinolytic method (see text)

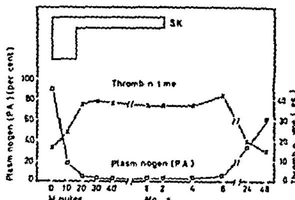


Fig 3 Plasminogen and thrombin time in a patient (A) treated with streptokinase for two hours

The maintenance dose in two patients was 150 000 units per hour by continuous infusion In the third patient this dose was reduced to 100 000 units per hour after six hours. Just prior to the initial dose 100 mg of hydrocortisone (Actocortin) was given intravenously and the treatment was followed by oral anticoagulant therapy No bleeding complication was seen and the Ivy bleeding time remained normal during the whole period of treatment in all patients

2 *Administration of urokinase*

The urokinase investigation was carried out by H Arnesen, M.D., in this laboratory The initial dose was about 250 000 units (3600 U/kg) in ten minutes and the maintenance dose the same amount per hour by continuous infusion. This treatment was also followed by anticoagulant therapy and no complications were seen

INVESTIGATION AND RESULTS

1 *Plasminogen determination in plasma containing various amounts of plasminogen*

The plasminogen level in plasma from a patient (C) one hour after beginning of the maintenance dose determined by a caseinolytic method was found to be 3.5 %. This plasma was mixed in various proportions with plasma collected from the same patient before treatment and the plasminogen levels were determined by the present clot lysis method The results are given in Fig 2 There is a good agreement between the calculated (abscissa) and the recorded (ordinate) values

2 *Plasminogen determination during streptokinase treatment*

Three patients (A B C) are presented and the results of plasminogen determinations and thrombin times are given in Figs 3 4 and 5 Very low

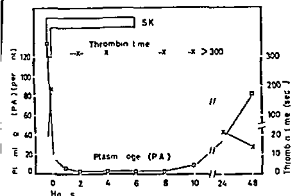


Fig 4 Plasminogen and thrombin time in a patient (B) treated with streptokinase for six hours

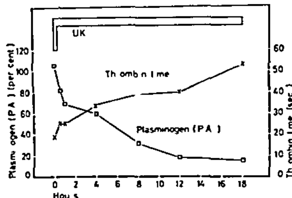


Fig 6 Plasminogen and thrombin time in a patient (D) treated with urokinase for eighteen hours

plasminogen values were recorded in all patients after the initial dose had been infused After 48 hours the plasminogen values had still not reached the pretreatment levels In patient B there was no detectable fibrinogen in the patient s plasma when the thrombin time exceeded 300 sec

3 *Plasminogen determination during urokinase treatment*

One patient (D) is reported and Fig 6 illustrates a different pattern of plasminogen decline than during streptokinase treatment

DISCUSSION

The caseinolytic method (16 21) has frequently been utilized for determination of plasminogen but may be less convenient in routine laboratories due to the necessity of highly standardized systems

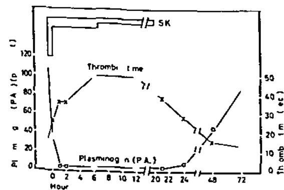


Fig 5 Plasminogen and thrombin tim in a patient (C) treated with streptokinase for twenty hours

(10 12) The application of fibrin plates (1) for this purpose (19) is not practical during thrombolytic therapy

Christensen (6) suggested a clot lysis method introducing bovine fibrinogen as substrate and human serum in combination with streptokinase as test material The mechanism was better under stood when Mullertz and Lassen (18) discovered a factor proactivator which reacted stoichiometrically with streptokinase in the formation of an activator of plasminogen The isolation of proactivator from human plasminogen has never been successful however and there is reason to be lieve that it is a property connected with the plasminogen molecule itself Plasmas of most ani mal species do not contain this proactivator property of plasminogen which makes them suitable as enzyme substrate in proactivator assays

Johnson and Tillett (14) Blomback et al (4) and Lassen (17) utilized this principle in clot lysis systems for the quantitative determination of plas minogen as proactivator The present method was based upon the same principle using bovine plasma instead of fibrinogen as substrate (2) It was found to be suitable for determination of plasminogen in fibrinolytic human plasmas probably giving the combined content of plasminogen and plasmin (3) Konttinen et al (15) found bovine plasma useful for such assays although in some respects it differed from purified fibrinogen On the other hand de Vreker (23) preferred fibrinogen in his modified system

The results obtained with the present method seem to be reliable giving a rapid answer with a high reproducibility when used in patients during

thrombolytic therapy In all the streptokinase treated patients plasminogen decreased to less than 6% of normal during the initial period and remained at this level to the end of therapy More than 48 hours was necessary to restore pretreatment values Plasminogen decline in the urokinase treated patient was much slower and the level never fell below 15%

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A NEW TECHNIQUE FOR DYNAMIC SPIROMETRY ON PATIENTS WITH CONTAGIOUS RESPIRATORY DISEASES

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Abstract A system with a disposable plastic bag in a box between the patient and the Bernstein spirometer is described which eliminates the risk of spreading infections between patients via the spirometer The present modification increased the amplitude response of the spirometer by about 3 at 100 c/min the random error was not increased

The analysis of the ventilatory function in patients with acute or chronic contagious diseases of the lungs or airways e g tuberculosis mycoplasma mycosis or purulent bronchitis involves some problems concerning disinfection of the spirometer Even if the spirometer is cleaned after each patient, which generally is not done it is not possible to guarantee a safe spirometer on all occasions without excessive labour for each cleaning procedure It is worse however if the infectious character of the disease is not suspected at the time of investigation and the spirometer therefore is not specially cleaned It is also unsatisfactory to refuse a patient from study until for instance a negative guinea pig inoculation test for tuberculosis has been obtained which may take up to six weeks There seems to be a need for a modification so that dynamic spirometry can be performed routinely on contagious patients without undue risks for other patients and undue labour for the laboratory personnel

In the present study a modification of dynamic spirometry is described in which the patients breathe in and out of a disposable thin walled plastic bag enclosed in a plexiglass cylinder which communicates with the spirometer The system has been studied in model experiments and tested on patients and is easy to use in routine practice

METHODS AND MATERIAL

The modification is described in Fig 1 Through a rubber mask and a largebore rubber tube (*A*) the patient breathes in and out of a thin walled (0 02 mm) plastic bag (*B* 40 × 50 cm effective volume about 13 l) attached by an O-ring to a groove in the metal cylindrical part *C* This siliconized O-ring seals the plastic bag airtight in a plexiglass cylinder (*D*) which communicates with a slightly modified Bernstein spirometer (*E*) (Kifa AB Solna) (1) The cone (*F*) prevents the air stream from the spirometer from turning the plastic bag inside out and causing an obstruction at *C* The tap (*G*) on top of the plexiglass cylinder helps to adjust the proper volume level of the spirometer

The plastic bag the rubber mask, the tube and the metallic part *C* are changed after each test all parts but the plastic bag being heat sterilized

The dynamic characteristics of the system were objectively tested by driving it with a 1 5 l pneumatic piston (E Bohlin Stockholm) at various frequencies The system was also tested on one group of normal subjects and one group of patients with obstructive pulmonary diseases The normal group comprised six men and nine women with an average age of 29 years (range 15–6?) The patient group consisted of ten men and one woman with an average age of 56 years (range 32–68) all with clinical signs of airway obstruction Both groups were studied twice on separate days half of each group starting with the normal spirometer the other half starting with the plastic bag and plexiglass cylinder inserted between the patient and the spirometer For each study three forced inspiratory and expiratory vital capacities were recorded and two maximal voluntary ventilations at each frequency For each measurement the highest value was used

Statistical calculations were performed according to Snedecor (7) The following probability (*P*) levels of significance were used $P < 0.01$ significant and $P < 0.05$* probably significant

RESULTS

The amplitude responses at varying frequencies of the spirometer with and without the present mod

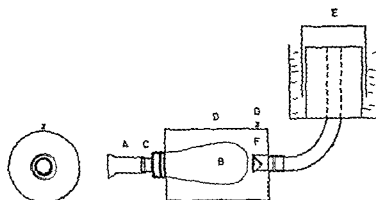


Fig 1 A modification of dynamic spirometry see text. *A* rubber mask and tube *B* plastic bag *C* metal cylindrical part with *O* ring for sealing the plastic bag in the plexiglass cylinder *D* plexiglass cylinder *E* Bernstein spirometer *F* cone *G* tap for volume control

Table I *Some ventilatory capacity tests and the difference between the values obtained with the present modification and with the spirometer alone (diff)*

P = probability that the differences are caused by random factors *C* = coefficiency of variation the error of a single determination in per cent of the mean value

	Control group $n = 15$				Patient group $n = 11$			
	Mean	Diff	*P*	*C*	Mean	Diff	*P*	*C*
VC	4 29	0 047	>0 0	2 5	3 18	0 013	>0 70	3 0
FEV 1 0	3 72	0 090	<0 05	2 8	1 54	0 017	>0 30	2.5
FEV	87	1 3	>0 05	2 2	47	0 5	>0 40	3.4
FIV 1 0	4 04	0 043	>0 30	3 3	2 69	0 043	>0 40	4.3
FIV	94	0 1	>0 70	1 1	84	1 3	>0 50	5.6
MVV 40	122	0 7	>0 30	1 7	54	0 1	>0 95	9.3
MVV 80	160	4 3	<0 05	3 2	62	1 3	>0 50	7.8
MVV$_F$	161	2 8	<0 01	1 3	63	1 3	>0 40	6.1

fication were tested with the pneumatic piston and are shown in Fig 1 With the spirometer alone the amplitude response increased successively with rising frequency the rise from 26 c/min to 126 c min being 13% With the present modification the corresponding amplitude increase was 19% at the frequency of 146 c/min At the frequency of 96 c min the amplitude increase with the spirometer alone was 6% and with the present modification 9% The distortion of the curves at higher frequencies is evident from Fig 2 slightly more marked changes were noted with the plastic bag attached

The effect of the present modification on the

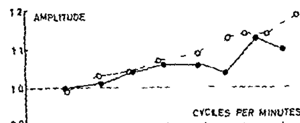


Fig Amplitude responses of the regular (●—●) and the modified spirometer (○—○) at varying frequencies when tested with a motor-driven 1 5 l pneumatic piston Each symbol represents the average of 10–20 consecutive cycles

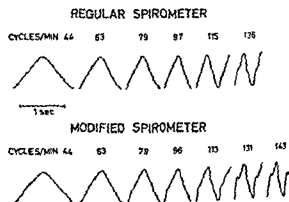


Fig 3 Volume curves at varying frequencies when tested with a motor-driven 1 5 l pneumatic piston

different variables of dynamic spirometry in the control group and the patient group is given in Table I The maximal voluntary ventilations were the only variables that were significantly changed being slightly larger when recorded with the plastic bag system These small absolute changes seem to be related to the increased amplitude response at higher frequencies as shown in Fig 1 As expected the coefficient of variation for the different variables was less in the control group than in the patient group

DISCUSSION

With the present technique of heat sterilization or exchange of all parts in contact with the expired air a safe spirometer can be guaranteed for each patient although used for subjects with tuberculosis mycoplasma mycosis or other infectious diseases This should be of special value in departments for infectious diseases and for tuberculous patients but also in hospitals with a mixed clinical material In routine practice the procedure with this modification takes only 2–3 min extra which is much less than any procedure for cleaning the spirometer

The present study shows that this modification gives a slight systematic error with a somewhat more pronounced distortion of the amplitude response at higher frequencies However at frequencies below 100 c/min and for practical purpose even up to 140 c/min this difference is quite negligible

The random error of the method as computed from the difference between the spirometric values obtained with and without this modification was in the control group (1–3 %) similar to the values given for dynamic spirometry by Berglund et al (2) but lower than those given by Ringqvist (5) In the patient group the error (3–9) was larger than the values given for patients with sarcoidosis (3) but less than those given for patients with bronchial asthma (4) and chronic bronchitis (6) Therefore no random error seems to be introduced with the present modification

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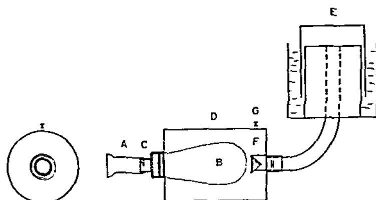


Fig 1 A modification of dynamic spirometry see text. *A* rubber mask and tube *B* plastic bag *C* metal cylindrical part with O ring for sealing the plastic bag in the plexiglass cylinder *D* plexiglass cylinder *E* Bernstein spirometer *F* cone *G* tap for volume control

Table I *Some ventilatory capacity tests and the difference between the values obtained with the present modification and with the spirometer alone (diff)*

P = probability that the differences are caused by random factors. C = coefficiency of variation the error of a single determination in per cent of the mean value

	Control group $n = 15$				Patient group $n = 11$			
	Mean	Diff	P	C	Mean	Diff	P	C
VC	4 29	0 047	>0 20	2 5	3 18	0 013	>0 70	3.0
FEV 1 0	3 74	0 090	<0 05	2.8	1 54	0 017	>0 30	2.5
FEV	87	1 3	>0 05	2 2	47	0 5	>0 40	3 4
FIV 1 0	4 04	0 043	>0 30	3 3	2.69	0 043	>0 40	4 8
FIV	94	0 1	>0 70	1 1	84	1 3	>0 50	5 6
VV 40	122	0 7	>0 30	1 7	54	0 1	>0 95	9.3
V 80	160	4 3	<0 05	3 2	62	1 3	>0 50	7 8
VV_F	161	2.8	<0 01	1 3	63	1 3	>0 40	6.1

ification were tested with the pneumatic piston and are shown in Fig 1 With the spirometer alone the amplitude response increased successively with rising frequency the rise from 26 c/min to 126 c min being 13 %. With the present modification the corresponding amplitude increase was 19 % at the frequency of 146 c/min. At the frequency of 96 c min the amplitude increase with the spirometer alone was 6 % and with the present modification 9 %. The distortion of the curves at higher frequencies is evident from Fig 2 slightly more marked changes were noted with the plastic bag attached

The effect of the present modification on the

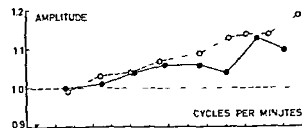


Fig 2 Amplitude responses of the regular (●—●) and the modified spirometer (O—O) at varying frequencies when tested with a motor-driven 1.5 l pneumatic piston. Each symbol represents the average of 10–20 consecutive cycles.

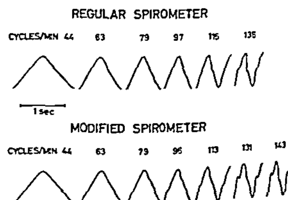


Fig 3 Volume curves at varying frequencies when tested with a motor-driven 1.5 l pneumatic piston.

Acta med scand Vol 186 pp 487–491 1969

IRON ABSORPTION IN APPARENTLY HEALTHY MEN AND WOMEN

III Studies in Iron Absorption[1]

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Abst act Thirty three women and ₋4 men all apparently healthy have been investigated in respect of iron absorption by means of rad oactive iron and a whole body counter Six out of 33 women had either a somewhat low serum iron concentration or a high TIBC and in nearly 40 of them absorption exceeded the upper limit for the men. The mean absorption of the women was about double that of the men No correlation could be shown to exist between absorption on the one hand and hemoglobin, packed cell volume serum iron and TIBC on the other

The introduction of the whole body counter into clinical medicine has simplified the study of iron absorption and has made it possible to carry out routine investigations of iron absorption as a link in the diagnosis of sideropenia intestinal disturb ances diseases of the blood etc Earlier methods of measuring iron absorption have been reported elsewhere (8) and have proved to be very la borious for clinical use

The aim of this investigation was to present values of iron absorption for both men and women of an apparently healthy condition A summary of so-called normal values previously reported is given in Table I

MATERIAL AND METHODS

Thirty three healthy women between the ages of 20 and 41 (mean age 26.5) and 24 healthy men between the ages of 21 and 50 (mean age 28 6) were stud ed In the histories of the persons invest gated there was no record of abnormal bleeding, anemia, menorrhagia or iron therapy during the year prior to investigation None of them had been a blood donor None had c nsulted a physician or received med cal treatment and a'l of them were working None of the women had been pregnant during the last year

The subjects were instructed to fast for 10 hours be fore and 2 hours after administration of the test dose In nine of the women hemoglobin concentration and packed cell volume were measured Twelve of the female subjects were investigated for serum iron concentration and total iron binding capacity (TIBC) and 21 only for serum iron In nine of the male subjects hemoglobin concentration and packed cell volume were investigated in five serum iron and TIBC and in seven only serum iron

Table II shows the normal values and the error in the determination of serum iron and TIBC performed at our own laboratory Serum iron was determined accord ing to Agner s method in which the protein-bound serum iron, after acidifying to pH 2.5–3 0 reacts directly with *o*-phenanthrolene without previous hydrolysis and pre cipitation of protein. The color intensity was subsequently determined colorimetrically (1) For TIBC determination, transferrin was first saturated with ferric chloride and surplus iron ions were precipitated by magnesium car bonate after which the transferrin bound iron was de termined as serum iron (13) From the serum iron values transferrin values can readily be deduced. At the begin ning of the investigation some serum iron and TIBC determinations were made at the Central Laboratory for Cl n cal Chemistry Karolinska Hospital

In order to reduce the effect of individual variations in the absorption of iron during the day the absorption tests were made between 9 and 9 40 a m

Iron absorption was investigated by means of radio-active iron 0 ₋5 mg of iron was gi en by mouth in the form of f rrous sulphate labelled with 0.5–1 μCi Fe^{59} in a small quantity of distilled water The beaker used for gi ing the test dose was rinsed 3 times w th distilled water which the subjects were asked to swallow so as to make c rtain that the full test dose was administered.

The radioactiv ty of the body was measured with the a d of whole body counter (11) This consists of a me suring chamber 2 0 205 ₋00 cm, built on a mount ng of low activity material which is known as *hofo s te* with walls of steel plate 15 cm thick The measurin in strument consists of sod um iodide crystals 5 3 inches

[1] Presented in part to the Panel of the Int rnational Atomic Ene gy Agency Vienna 1965

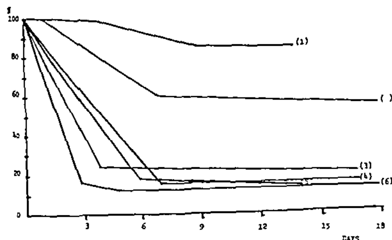


Fig 1 Whole body radioactivity after oral administration of 0 25 mg ferrous sulphate labelled with 0.5–1 μCi Fe⁵⁹ to two women (curves 1 2) and four normal men (curves 3–6) One of the women (1) had somewhat low serum iron

vestigation was made this value was taken as the basis for the changes in the body's radioactivity due to the radioactive material supplied

The whole body activity was measured prior to administration of radioactive iron and also 1 hour after

The dose retained after 13–20 days was regarded as absorbed, and it was assumed that excretion of already absorbed iron could be disregarded Measurement times and physical data are given in Table III

RESULTS

Repeated measurements of whole body activity on the days following the administration of the test dose showed that the values were greatly diminished during the first 3–4 days correspond ing to the activity eliminated from the body in the feces Fig 1 shows how approximately after 7 days the values become stabilized After 10 days hardly any of the test dose was excreted

Out of the 33 selected healthy women six had serum iron values below 0 070 mg or TIBC values above 0 400 mg%

In Table IV the results of the iron absorption investigation are given Substantial differences in iron absorption between women and men were obtained The higher absorption in women was found to be statistically significant ($0.01 > p > 0.001$) This applied also to the women whose serum iron values were above 0 070 mg and TIBC values below 0 400 mg%

Mean iron absorption in the women was 2 3 times higher than that in men which means that the women on an average absorbed 25 more of the given dose

Thirty six per cent of the women had a higher iron absorption than the man with the highest value which was 41% There was no statistically significant correlation between individual values of hemoglobin packed cell volume serum iron TIBC and iron absorption (Table V) Dispersion of the absorption values was so wide that even very low values were within the normal limits

DISCUSSION

Method

The low test dose of 0 25 mg was chosen because it was assumed to give rise to a physiologic amount of free iron ions in the small intestine

Values subsequently obtained were compared to the total radioactivity of the body after the administration of the test dose (In order to avoid

Table IV *Normal iron absorption (per cent of given dose)*

Subject category	No of subjects	Mean	S.E.
Male	24	19 0	2 3
Female entire group	33	43 5	4 4
Female serum iron > 0 070 mg TIBC < 0 400 mg%	27	39 7	4 5
Female serum iron < 0 070 mg TIBC > 0 400 mg%	6	60 5	1? 2

Table V *Correlation coefficients and regression coefficients between iron absorption and hematological variables in healthy fertile females*

Clinical variable	No of subjects	Correlation coefficient	Regression coefficient	Statistical significance of correlation
Hemoglobin concentration g/100 ml	9	−0.489	-3.24×10^{-3}	$p > 0.05$
Packed cell volume	9	−0.314	-3.98×10^{-3}	$p > 0.05$
Serum iron mg/100 ml	27	0.152	2.05×10^{-4}	$p > 0.05$
TIBC mg/100 ml	9	0.541	3.8×10^{-4}	$p > 0.05$

losses in the feces this initial value was measured after one hour)

The distribution of the isotope in the body during the time immediately after administration may cause the degree of efficiency to vary when measurements are made It has been shown that for a whole body counter with a one crystal and chair geometry 4 hours after administration the degree of efficiency is comparable to that obtained after 2 weeks (12 17) The dependence on geometry of the apparatus in which scanning geometry is utilized is however only between 1/4 to 1/10 of that of the apparatus in a chair geometry is applied (11) Therefore the initial values was measured one hour after administration of the test dose in the present investigations Even small losses of radioactive iron in the feces can then be avoided

If unabsorbed iron is excreted for more than 10 days after administration the method used may give too low absorption values After about 10 days however it seems that only 0.5 % of the administered dose is eliminated via the intestine (2) This also indicates that the amount of radioactive iron already absorbed which can leave the body in this way during the time the measurements are made must be very small although a certain excretory function with regard to iron has been ascribed to the cells of the intestinal mucosa (5)

On account of the short life cycle of the cells of the intestinal mucosa (2–4 days) (4) it is possible that iron taken up by these cells returns to the lumen of the intestine inasmuch as the cells are desquamated Whether this iron was absorbed is a semantic question (15) After 10–14 days when retention was measured the cells of the intestinal mucous membrane have probably been renewed once or twice and it is likely that the amount of iron due to the test dose which can be recovered in the mucosal cells is small

Results

Serum iron and TIBC values seem to be very widely dispersed normally (Table II) and the somewhat low serum iron and high TIBC values found in some of the women are not surprising They are also in good agreement with earlier reports in the literature (14 20) Single determinations of serum iron and TIBC thus seem to be of limited value as criteria of iron metabolism. Furthermore our investigations show that in most women there is an increased absorption from the gut of iron concentrations likely to occur physiologically This is in agreement with the recent reports by Heinrich et al (7) Since no correlation was found between any of the parameters hemoglobin packed cell volume serum iron TIBC and iron absorption intestinal absorption of iron seems to act independently of these factors The relation between such humoral factors and iron absorption have been further investigated and the results separately published (9 10)

ACKNOWLEDGEMENTS

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Acta med scand Vol 186 pp 493–497 1969

ON THE MECHANISM OF RENIN STIMULATION

The Effect of Postural Change Salt Depletion and Exercise

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Abst act The correlation between hemoconcentration (ex pressed by increase in colloid osmotic pressure (ΔCOP)) and plasma renin activity increase (ΔPRA) has been investigated during 1) change of posture from sup ne to standing position in normals, who were salt-depleted the three previous days (10 mEq Na per day on the third day 100 mg chlortalidone (Hygrotone®)) and 2) in exercise experiments on normals on liberal salt intake in sitting position on bicycle ergometer (medium work load–terminal pulse rate 140–140) The correlation ΔCOP–ΔPRA was in 1) linear and significant, $r = 0.83$ $p < 0.001$ The slope of the line was significantly steeper than in correspond ng experiments on liberal salt intake (PRA increase per unit COP increase was greater) Mean supine PRA was significantly higher than on liberal salt in take but the range overlapped In 2) a significant PRA increase in all experiments appeared but no corre ation ΔCOP ΔPRA. It is concluded that salt depletion of some days duration (chronic) sensibilizes the renin releasing mechanism for the postural stimulus The lack of cor relation ΔCOP–ΔPRA for the exercise stud es suggests a mechanism for renin stimulation d fferent from the one in postural shift and acute salt depletion

In a previous paper (11) the relationship b tween hemoconcentration (expressed by increase in colloid osmotic pressure (COP)) and increase in plasma renin activity (PRA) from supine to erect position in normal humans on liberal salt intake has been studied A significant linear cor relation between ΔCOP and ΔPRA was found Acute salt depletion (furosemide) of supine normals demonstrated a similar significant correlation The two regression lines were not significantly different Drawing of blood in vol umes (435 ml) corresponding to plasma volume decrease in the furosemide experiments from supine normals gave no increase in PRA It was concluded that PRA increase on chang of posture and in acute salt depletion was a function of hemoconcentration It was therefore found of interest to investigate the same relationship during changes of posture in salt-depleted normal individuals and during exercise in normals on liberal salt intake in order to study whether the renin release which is known to take place (3 4 and 8) could be explained in the same manner under these conditions

MATERIAL AND METHODS

Stud es on changes of posture during salt depletion were carried out in ten individuals aged 22–61 years six males and four females All were hospitalized for minor condi tions unrelated to the cardio-vascular or renal system. No drugs were given prior to the investigation

Studies during exercise on a b cycle ergometer were carried out in seven trained individuals aged 2–40 years, six males and one female Blood samples were obtained by placing an indwelling needle in an antecubital vein coagulation in the needle being prevented by flushing it with small amounts of 3 8% Na citrate Immediately before the blood samples were obtained a few ml of blood were drawn and discarded because of contamina tion with Na citrate from the need e Subsequently blood samples were drawn into two disposable syringes of 10 ml each containing 1 ml of Na citrate The blood was t ansferred to a sil conized Erlenmeyer flask im mersed into ice water W th n one hour the blood was centrifuged at 0 C 3000 rpm for 15 min. The plasma was separated and kept at −20 C until renin activity was determin d The renin activ ty was determined by the method of Boucher et al slightly modified (coefficient of variation ± 11 8%) (10) The results are expressed as ng angiot ns n/10 ml of plasma/4 h of incubation In addition to the blood obtained for measurements of renin activity another 3 ml of blood was drawn into a dry syring for determination of colloid osmotic pressure COP was recorded n an electronic osmometer for quick direct measurement of small samples described by Hansen (7) The results are expressed in cm H_2O Confidence limits 95 = ± 0 5 mm Hg Plasma volume in the stand

Table I *Postural experiments after salt depletion*

0=sample after 45 min of recumbency 5 20 and 30=min after assumption of the standing position
Mean standing values are obtained as average of 20 and 30 min values

Case no	Sex	Age (y)	PRA_0 / COP_0	PRA_5 / COP_5	PRA_{20} / COP_{20}	PRA_{30} / COP_{30}	Mean standing PRA / COP	Standing plasma volume in % of supine
1	♂	41	35	60	90	104	97	
			42 7	45 9	48 3	51 4	49 9	85 6
2	♂	31	84	125	177	169	173	
			51 2	59 4	59 0	57 7	58 4	87 7
3	♂	23	148	208	216	280	248	
			50 5	54 7	56 5	58 4	57 5	87 8
4	♀	?2	61		100		100	
			45 0	—	48 4	—	48 4	93 0
5	♂	22	58	58	112	132	122	
			44 9	48 6	49 5	50 7	50 1	89 6
6	♀	42	87	90	104	128	116	
			38 6	38 6	41 8	42 8	42 3	91 3
7	♀	36	54	55	100	90	95	
			43 1	42 8	47 5	48 5	48 0	86 9
8	♂	27	37	44	95	108	102	
			41 8	45 8	47 0	48 5	47 8	87 4
9	♂	53	57	61	133	128	131	
			38 6	41 8	43 6	45 0	44 3	87 4
10	♀	61	80		97	103	100	
			40 2	—	43 0	43 0	43 0	93 5
		Mean ± S E M	70 ± 10					Mean ± S E M
			43 7 ± 1 4					89 1 ± 0 9

ng position as % of the supine values was calculated as follows COP supine × 100/COP standing Plasma volume during exercise as % of the resting value was calculated in the same way

A Postural studies after salt depletion

A sodium free diet (10 mEq daily) was given for 3 days The third day 100 mg of chlortalidone (Hygroton®) was administered perorally in the morning The experiments were carried out on the fourth day between 8 and 10 a m. After 45 min in the supine position the patient was assisted to a standing position and blood samples were obtained at intervals as shown in Table I

B Exercise studies

Seven individuals carried out eight experiments on a bicycle ergometer After a resting period of 20 min on the bicycle saddle the first blood sample was drawn the other samples being taken at intervals as indicated in Table II The exercise performed for 30 min was moderately heavy (600–800 kg m/min) The pulse rate at the conclusion of exercise was between 120–140/min The experiment took place between 8 and 12 a m

RESULTS

A Postural studies after salt depletion (Table I)

The mean supine plasma renin activity was 70 ng angiotensin/10 ml plasma/4 h incubation ± 10 (S E M) which is significantly higher than on normal diet (normals on liberal salt ingestion, mean 15 ng ± 2 (12) $p < 0\,001$) Mean supine COP was 43 7 cm H_2O ± 1 4 which is significantly higher than on normal diet (normals on liberal salt ingestion mean 34 8 cm H_2O ± 0 6 (12) $p < 0\,001$) The increase in plasma renin activity from the supine to erect position is significant in all cases (the 95 % confidence limit of the method is exceeded) After 20 min of standing a plateau is reached in all experiments This increment is preceeded by a rise in COP in all cases After 20 min a plateau is attained in COP also These findings are the same as in postural changes on normal salt ingestion (11). Mean plasma volume in the erect position as % of the supine value is 89 1 which is significantly less than in corresponding investigations on normal sodium intake (normals 84 6 % ± 0 9 (12) $p < 0\,001$)

Fig 1 shows as abscissa the increase in CC and as ordinate the increase in PRA from supi position to 20 and 30 min of erect position correlation between ΔCOP and ΔPRA is 1 The regression line is $y = 13\,7x - 15\,2$ $r = 0$

Table II *Exercise*

0=sample after ?0 min of resting position on the bicycle 5 10 20 and 30=min after start of exercise Mean COP during exercise is obtained as average of 20 and 30 min values Work load is indicated in kg m/min The two experiments performed in case 3 are indicated as 3*a* and 3*b*

Case no	Sex	Age (y)	PRA / COP	PRA_5 / COP_5	PRA_1 / COP_{10}	PRA_{20} / COP	PRA_{30} / COP_{30}	Work load	Mean COP during exercise	Plasma volume during exercise as % of resting plasma volume
1	o	24	21	40	—		43			
			39 9	44 3	43 0	—	44 2	900	44 2	90 3
2	♀	22	43	39	64	69	71			
			38 5	40 1	41 5	44 0	43 3	600	43 7	88 1
3*a*	♂	28	40	36	39	58	54			
			42 6	44 0	44 7	43 7	44 8	900	44 3	96 2
3*b*	♂	28	39	68		60				
			45 8	45 1	—	44 6	—	700	44 6	10? 7
4	o	30	20	40	40	76	76			
			45 9	46 6	47 8	46 5	46 7	700	46 6	98 3
5	♂	34	30	50	56	—	60			
			42 3	46 0	46 7	46 5	47 5	600	47 0	90 0
6	♂	23	48	51		79	85			
			42 6	42.6	—	41 6	43 0	700	42 3	100 7
7	♂	40	8	12		28	37			
			41 9	44 0	—	44 5	45 5	600	45 0	93 1
										Mean±S.E.M. 95 0±1 9

$p < 0\,001$ The corresponding regression line for normals on liberal salt intake (11) is drawn in Fig 1 as well The slope of the line of salt depleted persons is significantly steeper ($p < 0\,001$)

B *Exercise studies* (*Table II*)

The increase in PRA from resting to exercise values is significant in all cases A plateau is already reached after 5 min of exercise in cases 1 3*b* and 5 and is found in all cases after 20 min Though an attempt was made to maintain equally heavy exercise for all which seems to have been achieved (small differences in pulse rate at the end of exercise) the COP rise varied from experiment to experiment In some of them it did not rise at all (cases 3*b* 4 and 6) The greatest increase corresponded to an exercising plasma volume of 88 1 / of resting plasma volume Mean exercising plasma volume as of "resting" plasma volume is 95 0 which is significantly less than in the corresponding postural study ($p < 0\,001$) In all experiments in which COP did rise a plateau was attained in the blood samples drawn after 5 min of exercise except in case 2

Fig 2 shows the increase in COP from resting values to values after 20 and 30 min of exercise as abscissa and the increase in PRA in corresponding samples as ordinate The correlation ΔCOP–ΔPRA is not significant The correlation line is calculated by the method of least squares $y = -1\,9x + 34\,5$ $r = -0\,31$ ($0\,30 < p < 0\,40$)

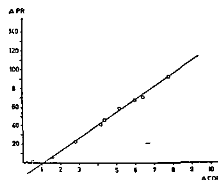


Fig 1 Postural experiments Normal individuals after 3 days of salt depletion Correlation between increase in plasma renin activity (ΔPRA) and increase in plasma colloid osmotic pressure (ΔCOP) from resting values to values after 0 and 30 min of standing The dashed line is a demonstration of the regression line of normal individuals on liberal salt intake

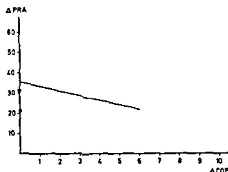


Fig 2 Correlation between increase in plasma renin activity (ΔPRA) and increase in plasma colloid osmotic pressure (ΔCOP) from resting values to values after 20 and 30 min of ergometer exercise

DISCUSSION

In the postural studies carried out after salt depletion a linear correlation between ΔCOP and ΔPRA was found but the slope of the regression line was steeper which may be interpreted as a sensibilization of the stimulus of renin release caused by the sodium depleted state (PRA increase per unit COP increase is greater) A similar sensibilization has previously been re[illegible] by Bunag et al (1) who demonstrated a [illegible]tated renin response to constriction of a [illegible] artery in chronic salt depleted dogs If such a sensibilization took place during the course of acute salt depletion the ΔCOP–ΔPRA relationship would not be rectilinear but curvilinear As there are no indications of this (11) it is conceivable that a chronic salt depletion (lasting some days) is necessary to produce this effect There is a certain overlapping between the range of supine PRA for salt depleted individuals (35–148 ng) and the corresponding range for individuals on liberal salt ingestion (0–36 ng) but the relationship ΔCOP–ΔPRA follows different regression lines This suggests that the normal range of supine PRA is not greatly influenced by various salt ingestion

In the exercise studies no correlation was found between ΔCOP and ΔPRA suggesting a different mechanism of stimulation Castenfors (3) has reported that the renin increase during exercise in rats could be prevented by ganglion blocking agents therefore concluding that the autonomic nervous system is of importance to renin release He points out the possibility of a direct sympathetic stimulation of the juxtaglomerular apparatus because treatment with dihydralazine minimizing the renal vasoconstriction did not influence the renin response Bunag et al (1) have found the sympathetic system of significance for renin release in bleeding dogs as ganglion blocking and local anesthesia of the kidney nerves inhibited a rise in renin Other investigators have found similar evidence for a role of the sympathetic system in renin stimulation (5 14 15) On the other hand we know that the homotransplanted human kidney reacts to salt depletion and changes in posture by renin increase as in normal individuals (6 9) Vander and Luciano (13) have also found that renin increase in acute sodium depleted dogs may take place although the sympathetic nervous system has been thoroughly blocked by surgical denervation of the kidney local anesthesia of the kidney hilus and alpha and beta blocking agents The author mentions the possibility of an unknown humoral factor as stimulator in this situation The quoted studies point to the presence of a neural and a non neural (humoral?) stimulating mechanism of the renin system Our investigations are compatible with this concept It is suggested that Vander s humoral factor may be some function of hemoconcentration (viscosity increase COP increase or both)

ACKNOWLEDGEMENTS

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ABNORMAL GROWTH HORMONE RESPONSE TO INGESTION OF GLUCOSE IN JUVENILE DIABETICS

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Abst act Usin a chromatographic radio immuno ogical method serum growth hormone has been determined in thirteen controls and six newly diagnosed untreated juvenile diabetics durin a six hour oral glucose toler ance test under strictly standardized conditions The con trols had a mean peak value of serum growth hormone five hours after glucos ingestion while the juvenile diabetics exhibited an early hyperresponse in serum growth hormone with a mean peak value 2 / hours after glucose No difference could be demonstrated be tween fastin serum growth hormone in the two groups These resu ts are discussed in the light of the hypothes s of Rabinowitz and Zierler and of Youn

The role of growth hormone in intermediary metabolism and esp cially in the homeostasis of blood sugar has b en well known for many years on the basis of experiments in animals and studies in human subjects (6 11 15) It has been sug gested that an abnormality of growth hormone se retion may be of importance in the pathogenesis of human diabetes mellitus (37) How ever serum growth hormone has not b en shown to be abnormal in diabetic patients

Since the advent of the radio immunological principle for plasma hormone de ermination our knowledge of the dynamics of growth hormone has been very much extended Roth et al (25) have described fluctuations in plasma growth hormone during oral glucose loading a primary suppression 1–3 hours after glucose followed by an increase in the p asma concentration of growth hormone 4–5 hours after glucose They also showed that insulin hypoglycaemia or a drop in intracellular glucose after administration of 2 deoxy glucose is accompanied by a significant rise in plasma growth hormone

Plasma growth hormone has been found to be normal in fasting diabetics (8 13 36) The pos sibility exists however that the factors and mechanisms regulating growth hormone secretion are altered in diabe ics This problem has been studied extensively in maturity-onset diabetes but no significant abnormality has been found (13 34) with the exception of the growth hormone response to arginine (17 30) which is found to be depressed in female patients The clinical picture and the biochemical pattern (including plasma insulin) of juvenile diab tes differs in many respects from that of maturity-ons t dia betes However very little attention has been paid to plasma growth hormone in this condition

The following is a report of the results of serum growth hormone determinations using a chromatographic radio immunological method in a group of untreated newly diagnosed juvenile diabetics Growth ho mone was determined in the fasting state as well as during an oral glucose load with 100 g of glucose

MATERIAL

Diab tic s bjects

Six patients with newly diagnosed untreated d abetes mellitus of the juven le type admitted to the Second Un versity Clinic of Internal Med cine Kommunehospi talet, Aarhus The a e of the pat ents ranged from 10 to 33 years with a mean a e of 19 years Fi e of the s x patients had intermittent or permanent ketonuria The fast n blood sugar on the day of examination rang d between 147 and 301 mg per 100 ml w th a me n fasting blood sugar va ue of 20? mg per 100 ml

[1] *P e ent add ess* Aarhus Amtssygehus Aarhu D n mark

nc twelve subjects examined after repeated un tures or venous cathe erization

The average fasting serum growth hormone nd in the small group of juvenile diabetics s nted here is in agreement with other studies ving that juvenile diabetics have a normal fast c serum growth hormone level (1 20)

However during a six hour glucose tolerance e t an early hyperresponse in growth hormone ecretion was observed in juvenile diabetics This bnormality has not been described before

Parker et al (20) measured the growth hormone esponse to a glucose load in 14 diabetic children and 15 normals of the same age They concluded that there was no statistically significant differ nce between the growth hormone response to glucose in diabetic and normal children However scrutinizing their table it does seem as if there is a tendency to an earlier rise and to a broadening of the peak in the diabetics The severity of the diabetes in the individual cases seems to have varied greatly as the admission blood sugar ranged from 83 to 770 mg per 100 ml This pronounced scatter may explain the lack f a sta is ically significant difference

The study of Baker et al (1) on the me hanism partial remission in juvenile diabetics contains plasma growth hormone values obtained during an oral glucose load in ten untreated juvenile dia betics and ten normal children In non-diabetics they found in agreement with all o her in vestigators a growth hormone peak about 4 to 5 hours after glucose Surprisingly the plasma growth hormone curves after glucose in the dia betics showed no significant change at any time during the test

The mean curve from our six cases shows a peak at 2 $^{1}/$ hours after glucose This peak is significantly higher than the lowest value found during the glucose load The early high value found in a patient (J N) already $^{1}/_{4}$ hour after glucose ingestion might have been caused by sampling procedures as also in control no 9 as discussed above

The abnormal growth hormone response to glucose in juvenile diab tics demonstrated in the present study may be compared with the results obtained recently by Prange and Johansen (21) in this laboratory Studying blood sugar NEFA serum insulin serum growth hormone and serum glucagon every 30 minutes for a 24 hour p riod of daily life they observed that growth hormone peaks occurred more often and that they were higher in juvenile diabetics than in non-diabetics Some of the peaks in diabetics occurred just after meals and without relation to blood glucose or NEFA

The late rise in plasma growth hormone in normal individuals is usually explained as a response to tissue starvation which results in a suppression of glucose uptake in the muscle and in the release of fatty acids from the depots

Glucose uptake in muscle is very low or zero in the fasting state and also during glucose loading in untreated juvenile diabetics as has been shown by Butterfield and Wichelow (3) and by Ørskov and Christensen (38) Assuming that the same is true as regards fat tissue the other large metabolizing area in the body there seems to be very little or nothing for growth hormone to suppress in juvenile diabetics

It might be however that the early rise of growth hormone in juvenile diabetics observed in the present study is not of the same nature as that found in non diabetics Perhaps it should be compared to the unexplained plasma growth hormone peaks occurring now and then during a 24 hour period (8 14 18 22 29)

Rabinowitz and Zierler (23) have proposed a hypothesis to explain the pattern of plasma insulin and growth hormone in the fasting state and after food intake They suggested a three phase cycle the first phase starting immediately after eating the second phase two to four hours after eating and the third later than four hours after eating The first phase is charact rized by a high plasma insulin and a low plasma growth hormone concentration in the second phase the plasma insulin concentration is still high but plasma growth hormone is increasing in the last part of the phase the third phase is dominated by a high plasma growth hormone and a low plasma insulin concentration This mod l fits well with our concepts of carbohydrate protein and fat metabolism during and after a meal in the normal individual

If in some condition e g a geneti abnormality the growth ho mone response to glucose was changed towards an earlier p ak a disproportion between the available amounts of insulin and growth hormone in the b ginning of the second phase of Rabinowitz and Zierler

would obtain requiring a higher insulin production to assure normal metabolic events Such a stress of the beta cells of the pancreas might finally result in an exhaustion of the pancreas leading to diabetes mellitus

It is well known that no rise in plasma insulin occurs in classical juvenile diabetes after administration of glucose This fact together with the early growth hormone peak observed in the present study may be explained as the end result of the hypothetical series of events described above and thus could fit into Young s theory about the role of growth hormone in the pathogenesis of diabetes mellitus For this explanation to be acceptable prediabetic subjects should have the same early growth hormone peak but the insulin response to glucose should be augmented

Immunologically detectable plasma insulin was determined during oral glucose loading in prediabetic subjects by several authors The results obtained in these studies are however conflicting Grodsky et al (10) found a normal insulin response in prediabetics Ricketts et al (24) found significantly elevated plasma insulin one and two hours after glucose while Colwell and Lein (4) and Shima and Foa (27) found a tendency to a delayed hyperresponse in plasma insulin after oral glucose Plasma growth hormone results obtained during oral glucose loading in prediabetic subjects are also controversial To our knowledge only three investigations exist two of which are published in abstract form only (28 31) while the third deals with the growth hormone response to glucose in a family with heavy diabetic disposition (16) Unger et al (31) found a significantly higher plasma growth hormone response 4 and 5 hours after the glucose in prediabetics than in the controls Levine and Recant (16) found an identical growth hormone pattern in a prediabetic group and a control group In a recent publication Sonksen et al (28) described the growth hormone pattern in 18 male prediabetics after an oral glucose loading They found the usual late rise 4 to 5 hours after the glucose in both the prediabetic and control group but seven of the 18 prediabetics exhibited also an early rise 15 to 45 minutes after the glucose compared to three of the 21 controls and the plasma growth hormone values obtained 15 30 45 and 60 min after glucose were significantly elevated in the prediabetic group

In a study of the carbohydrate metabolism of patients with Turner s syndrome (18) we have found an abnormal glucose tolerance in six out of ten cases In four of these cases serum insulin and serum growth hormone were studied after a glucose load These patients with chemical diabetes showed an early peak in serum growth hormone Serum insulin rises as in normal persons but remains high for several hours

Another indication of an abnormality of growth hormone secretion in the prediabetic phase appears from a recent publication by Boden et al (2) They reported a slightly increased fasting plasma growth hormone as well as a hyperresponse to tolbutamide in genetically prediabetic patients

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INFLUENCE OF GASTRECTOMY ON LEUCOCYTES IN BLOOD DURING EXERCISE IN TUBERCULOUS PATIENTS

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Ab t act The leucocyte count of capillary finger blood determined at rest and during a 30 min exercise period in 13 gastrectomized and 9 non gastrectomized patients with pulmonary tuberculosis is reported

The increase with exercise of polynuclear and of total number of leucocytes was less ($p < 0.05$) in the group of gastrectomized pat ents No relationships of significance could be observed between different absorpt on tests and the increase of leucocytes in blood with exercise in this material

The number of leucocytes in blood at rest and during exercise was correlated ($p < 0.05$) with the concentration of gammaglobulins in blood and the tuberculin reactivity of the patients those with weak reactivity and low gammaglobulin concentrations and low leucocyte values

An increased incidence of tuberculosis in patients with gastrectomy has been observed in repeated studies (2 3 7 16 18) This has been attributed to the nutritional state of these patients since a correlation between tuberculosis and poor nutri tion has been found (10)

The mechanism for this increased morbidity with nutritional deficiency is not known but some animal and clinical experiments suggest that an insufficient supply of proteins (14) or amino acids (11 15 22) and vitamins A and C (9 11 15) could be a causal factor It has been suggested that patients suffering from gastric insuffic ency have an impaired cellular defence expressed as absence of leucocytosis in febrile infections (8)

As the leucocytes are part of the body s defence mechanism against infection it was considered appropriate to investigate whether or not a rela tionship between nutritional factors and amount of leucocytes in blood could be observed

The amount of leucocytes in blood at rest however is not representative of the total number of leucocytes in the body During stress as after adrenalin injection (17) or during exercise (1) high leucocyte counts in blood have been re corded which has been attributed to a redistribu tion of leucocytes from other parts of the body The amount of leucocytes in blood during a standardized exercise test might therefore be a better variable to study than the amount of leu cocytes at rest

MATERIAL

The patients in this study were all included in a larger investigation (10) in which the material is described in greater detail and more clinical data are given All patients were hospitalized because of their pulmonary tuberculos s One group (OP) consisted of 13 patients with previous gastrectomy (one B I twelve B II) the other group (NO OP) of nine patients with only pul monary tuberculosis

The mean extent of tuberculosis was the same in both groups Patients with cardiovascular diseases or abnormal ECGs at rest or during an exercise test were not accepted Furthermore only patients who after the preliminary exercise test were considered fit for a 30 min exercise period were included in the two groups

METHODS

Apart f om record ng of routine clinical data such as tuberculous reactivity e tent of tuberculos s, bacillary occurrence and resistance some special malabsorption tests were also performed (fo details see (10)) The following nutritional variables were recorded serum fol c acid activity v tamin A serum concentration and absorption radio-B absorption vitamin B_{12} concentra tion serum iron concentration and absorption serum proteins and serum chole terol

The f rst work test consisted of a standardized exercise test (0) with recording of heart rate electrocardiogram

Table I *Some data obtained at rest or during two exercise tests in 13 gastrectomized and 9 non gastrectomized patients with lung tuberculosis*

Mean values and standard errors of the means are given and the significance of the differences between the groups

	I Gastrectomized	II Non gastrectomized	Difference I II	P level
Age y	57 1 ± 2 0	48 0 ± 2 2	8 9	< 0 05
1st test				
W_{150} kpm min	565 ± 42	672 ± 47	−107	n s
W_{m} kpm min	54? ± 45	733 ± 41	−189	< 0 05
2nd test				
Work load kpm min	388 ± 35	555 ± 38	−167	< 0 05
Heart rate beats/min	144 ± 4 7	146 ± 4 1	− 2	n s
Work load W_m	0 72 ± 0 02	0 75 ± 0 0?	− 0 03	n s
Leucocytes mm³ At rest	5985 ± 467	5589 ± 3?8	396	n s
Leucocytes mm³ Max value during exercise	9119 ± 742	9689 ± 742	−570	n s
Leucocytes mm³ After 30 min exercise final value	7965 ± 851	9?33 ± 738	−1268	n s
Leucocytes increase with exercise final to resting value	40 0 ± 9 0	67 7 ± 5 9	− 27 7	< 0 05

and respiratory rate during stepwise increased work loads on a bicycle ergometer (12) As most subjects were exhausted at heart rates well below 170 beats/min the work load at heart rate (W) was determined instead of W_{170} as well as the work load at maximal working intensity (W_m) (?3)

The second work test consisted of a 30 min exercise period in sitting position at a work load (W) of about of W_m Both W W_m and heart rate after 30 min exercise were used as variables reflecting the relative work load The leucocytes in capillary finger blood were determined in duplicate at rest in supine position before the exercise and thereafter every 5 min during the exercise period

For leucocyte counts standard methods were used as described by Ahlborg (1) Detailed differential count was not performed, but polynuclear and mononuclear corpuscles were determined separately All blood samples were taken and prepared by the same nurse She also performed all the cell counts

In seven patients the leucocyte count was simultaneously determined in arterial blood samples There was no significant difference between the arterial and the capillary blood samples ($p > 0\,05$) Considering these two samples as double determinations the standard deviation for a single determination for leucocytes in blood at rest or during exercise was ± 9.5%

Statistical calculations were made according to Snedecor (21) The following probability (P) levels of significance were used $p < 0\,001$ highly significant $p < 0\,01$ * significant and $p < 0\,05$* probably significant. Regression and correlation analyses were performed by the method of least squares on an IBM 360 computer

RESULTS

Some data from the gastrectomized group and the control group are shown in Table I On the average the gastrectomized patients were older

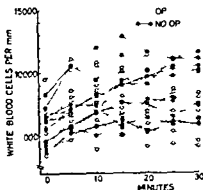


Fig 1 Leucocyte counts in blood at rest and during sitting exercise in 13 gastrectomized (OP) and 9 non gastrectomized (NO OP) patients with pulmonary tuberculosis.

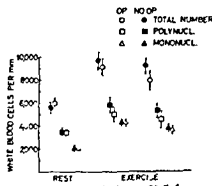


Fig 2 Mean values ± standard error of the means for leucocytes at rest and during exercise Symbols as in Fig 1

and performed a lower maximal work load in the first test and a lower work load during the second test when the leucocytes were counted Age (range 37–70 y) work load (200–750 kpm/min) heart rate after 30 min exercise (123–174 beats/min) maximal work load (300–900 kpm/min) and the work load in per cent of the maximal work load (58–88) did not significantly influence the leucocyte counts during exercise in this material As was expected the 30 min work load the heart rate at this load and the maximal work load decreased with rising age

The individual values for leucocytes at rest and during exercise are given in Fig 1 The average values at rest and peak and final values during exercise of total number of leucocytes polynuclear leucocytes are given in Fig 2 There was no difference in absolute numbers between the two groups

Peak values for total leucocytes were reached after 25 min exercise in the control group and after 19 min in the gastrectomized group the difference not being significant The relative increases of leucocytes with exercise are given in Fig 3 The percentage increase in both total number of leucocytes and in polynuclear leucocytes from rest to the end of the work test was significantly less in the gastrectomized group ($p <$ 0 05)

When the absolute values of the leucocyte counts at rest and during exercise as well as the relative increases with exercise were related to the clinical data there were no relationships of even

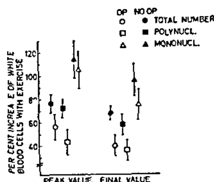


Fig 3 Mean values for increase of leucocytes during exercise Symbols as in Figs 1 and 2

probable significance with the nutritional and absorption tests The only data that were related to the leucocyte counts were the tuberculin reactivity and the amount of gammaglobulin in blood These relationships are given in Table II The patients with weak tuberculin reactions had lower leucocyte counts at rest and during exercise than those with marked tuberculin reactions Patients with low proportion of gammaglobulin in blood had lower leucocyte counts during exercise than those with high gammaglobulin values

DISCUSSION

Active forms of tuberculosis like other infections result in a more or less pronounced rise of leucocytes This is interpreted as a sign of an intact defence mechanism of the body It has been sug

Table II *Relationships between some clinical data and different leucocyte variables in 22 patients with pulmonary tuberculosis*

The significance of the regression coefficients (b/ε) is given

Independent variable (x)	Dependent variable (y)	b/ε
Tuberculin reaction ($n=22$)	Leucocytes at rest	−2 90
	Leucocytes max value during exercise	−2 23
	Leucocytes after 30 min exercise	−2.53
	Polynuclear leucocytes max value during exercise	−2 71
	Polynuclear leucocytes after 30 min exercise	−2 55
Gammaglobulin in blood mg/100 ml ($n=15$)	Leucocytes max value during exercise	2 31
	Leucocytes after 30 min exercise	2.90
	Increase of leucocytes from rest to max value during exercise	2 54
	Polynuclear leucocytes max value during exercise	2 76
	Polynuclear leucocytes after 30 min exercise	2 94

Tuberculin reaction classified as follows 1) negative to 1 mg of OT 2) positive to 1 mg of OT 3) positive to 0 1 mg or more of OT 4) positive to 0 01 mg or more of OT

gested (8) that febrile infections in patients with gastric insufficiency are not infrequently associated with leucopenia, or at least the absence of leucocytosis due to an impaired cellular defence

The gastrectomized tuberculous patients in the present study did not increase their leucocytes during the extended physical stress as much as the non gastrectomized This is in agreement with the earlier suggestion (8) It is not likely that this difference between the groups was caused by the lower mean work load and higher mean age in the gastrectomized group as no effect of age per se on the increase of leucocytes with exercise could be observed within the present material and no effect of the relative or absolute work load

The observed increase of leucocytes in blood by about 50 % after 30 min of exercise is far above the expected 10 % of hemoconcentration due to the exercise (5) and can thus not be ascribed to this hemoconcentration alone In a group of young normal men the increase of leucocytes with exercise was found to be best correlated to work time but also significantly to absolute and relative work load (1) The increase of total number of leucocytes with exercise was in the present study of elderly patients similar to the increase in the group of young men although the absolute work load was less As the heart rates were similar however the relative work load was on the average probably higher for the elderly patients with an expected higher maximal heart rate In the present study the number of mononuclear leucocytes increased more with exercise than the number of polynuclear In the group of normal young men (1) the opposite was the case but whether this difference should be attributed to the disease cannot be evaluated at present However a lymphocytic phase of leucocytosis with exercise has previously been described, preceding a neutrophilic and a toxic phase (4 13)

Though there is a correlation between tuberculosis and postgastrectomy state this does not necessarily mean that the low leucocyte response to the infection is impaired by malnutrition In the present study no relationship between the amount of leucocytes and the nutritional or absorption test used could be demonstrated

Folate deficiency with hypersegmented polymorphs has been found in tuberculous patients in England (19) but not in Sweden (6) However folate deficiency is frequently found in gastrectomized tuberculous patients (6) and in this study five of the gastrectomized patients had subnormal folate values versus one patient in the non operated group Evidently this deficiency does not seem to influence the amount of leucocytes The lack of leucocyte response to physical stress and the impaired nutritional state in gastrectomized patients may be two independent factors in the malabsorption syndrome However it has to be emphasized that the number of patients was small and the list of nutritional and absorption tests used in this study was not complete There may be other variables to investigate e g amino acids that may be better related to the general protein synthesis The correlation found between low gammaglobulins and low leucocyte counts may also be attributed to a low protein synthesis in general The role of lymphocytes for gammaglobulin production is well known but a correlation between low mononuclear counts and gammaglobulin could not be found in the present investigation

The patients with low leucocyte counts also had low tuberculin reactivity The simultaneous lack of both these diagnostic signs in patients with active extended tuberculosis with bacilli may have to be considered in the single case

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THE EFFECT OF MORPHINE ON ARTERIAL BLOOD GASES IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Abstract The arterial blood gas state was examined before and after an intramuscular injection of 1 cg morphine chloride in ten patients with uncomplicated acute myocardial infarction Before morphine the CO_2 tension was normal, whereas the O_2 tension was definitely but moderately reduced After morphine there was a slight tendency to an increase in the CO_2 tension associated with an even smaller further decrease in the O_2 tension Two patients with clinical signs of moderate pulmonary congestion showed the greatest blood gas disturbances both before and after morphine The data gave no evidence that morphine caused further impairment of tissue oxygenation

Recently there has been some discussion about the place of morphine in the treatment of acute myocardial infarction but relevant clinical studies are few Thomas et al (8) studied the hemodynamic effects of intravenous injection of morphine in patients with acute myocardial infarction and concluded that the circulatory response to morphine was very variable and not predictable However they found no consistent changes in the arterial blood gases

Kirby and McNicol (1) studied patients with acute myocardial infarction and found a high mortality rate in patients with uncompensated metabolic acidosis They suggested that care be taken with any procedure such as the administration of morphine that might interfere with the compensatory pulmonary hyperventilation

The need for further investigation in this field has led to the present study on the effects of intramuscular injection of 1 cg of morphine chloride on the arterial blood gases in patients with uncomplicated acute myocardial infarction

MATERIAL

Ten patients nine men and one woman aged 40 to 68 years (mean 57 7) with acute myocardial infarction were studied 1 to 48 hours after the onset of symptoms All were in normal sinus rhythm none had arterial hypotension severe pain or Cheyne-Stokes respiration Two patients had persistent basal pulmonary crepitations, none of the other patients showed any evidence of pulmonary congestion None of the patients had been given morphine or any medication known to influence the cardio-pulmonary function prior to the start of the study The patients had no oxygen for at least two hours before the start of the study

METHOD

The patients were studied in the supine position in bed with the head slightly raised breathing air Arterial blood was withdrawn through a polyethylene catheter in the femoral artery inserted at least 15 min before the start of the study After collection of two control samples taken 10 min apart 1 cg of morphine chloride was injected intramuscularly then in the course of the following 60 min a further six samples were withdrawn at 10 min intervals

Throughout the study blood pressures were recorded by a sphygmomanometer on the right arm and notes on the patients clinical condition were taken The patients were not allowed to fall asleep

The oxygen tension (PO_2) and the carbon dioxide tension (PCO_2) were measured by means of an Instrumentation Laboratory Inc Gas Analyzer Model 113 and pH was measured on a Radiometer pH meter Model 27 and a Radiometer Micro Electrode Unit The hemoglobin oxygen saturation (SO_2) was read from a Radiometer Blood Gas Calculator (6) the correctness of the readings was frequently checked by direct spectrophotometric determinations with a Beckman Spectrophotometer Model DU according to Refsum (5)

RESULTS

The results are shown in Fig 1 Many of the patients showed considerable variations in the blood gases during the observation period both before and after the injection of morphine

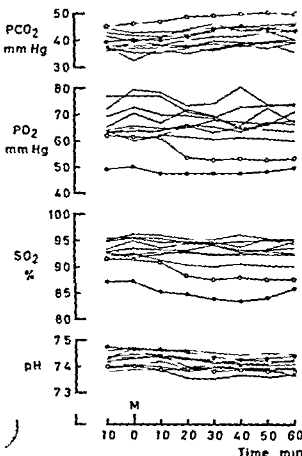


Fig 1 The effect of 1 cg morphine chloride i m. on the arterial PCO PO, SO and pH in ten patients with acute m ocard al infarction. M indicates injection of morphine The curves marked by open and filled circles represent two patien s with mild pulmonary congestion.

Before Morphine

Clinical

Some patients complained of slight chest pain but the majority were without symptoms Two patients had some crepitations over the bases of the lungs posteriorly

Blood pressure

One patient had a blood pressure of 215 140 and two others had moderate systolic hypertension The rest of the patients had normal pressures

Blood gases

The two patien s with pulmonary congestion showed the most pronounced deviations from normal When these two patients are excluded and the mean of the two sets of observations is taken as representative for the single patient it appears that PCO was within the normal range (35–45 mm Hg) while PO was below the lower limit of normal for the relevant age groups (80–75 mm Hg) in all patients SO was at the lower limit of normal (95 %) or below However neither PO nor SO were markedly reduced all values of PO_2 being higher than 60 mm Hg and of SO above 91 0 % pH was generally in the high region of the normal range (7 35–7 45) The averages were PCO 38 5 mm Hg, PO 68 3 mm Hg SO 93.9 % pH 7 426

After Morphine

Clinical

In all but one patient morphine caused drowsiness and/or relief of pain—from 10 to 20 min after the injection One patient complained of short lasting nausea 4 min after the injection of morphine but there was no accompanying change in blood pressure In the two patients with crepitations over the bases of the lungs the auscultatory findings were not altered after the administration of morphine within the observation period

Blood pressure

Of the three hypertensive patients two showed a moderate fall in systolic blood pressure otherwise there were no changes in blood pressure

Blood gases

The two patients with pulmonary congestion showed a clear tendency to further impairment of the blood gas picture particularly with regard to PCO and SO However in the most hypoxic of the two a marked increase in PCO from 39.5 to 45 0 mm Hg was associated with a very small further decrease in PO from 50 to 47 5 mm Hg The other patient with pulmonary congestion showed a rise in PCO from 46 0 mm Hg to 50 0 mm Hg and a fall in PO from 61 5 mm Hg to 54 5 mm Hg. In these two patients SO decreased from 87 2 to 83 4 % and from 91 5 to 87 5 % respectively In the remaining patients who did not have pulmonary congestion there was a slight tendency to increase in PCO and decrease in pH but no obvious changes in PO and SO The maximal changes of the average figures were found 30 min after the injection for PO and SO and 50 min after the injection for PCO and pH the

averages at these observation times being PCO_2 ... 0 8 mm Hg, PO_2 67 1 mm Hg SO_2 93 2 % pH 7 404

DISCUSSION

The data show that uncomplicated acute myocardial infarction is usually associated with a fairly normal PCO_2 and a clearly though moderately reduced PO_2 in arterial blood This demonstrates that there is generally no major change in the alveolar ventilation but a definite disturbance of the alveolar/arterial gas exchange This is in agreement with the observations of others (2 3 4 8) McNicol et al (3) and Valentine et al (9) who studied the effect on PO_2 of 100 % oxygen breathing claimed that the hypoxemia was mainly due to ventilation/perfusion disturbances and could only to a minor degree be ascribed to true anatomical shunting while MacKenzie et al (2) and Pain et al (4) using the same approach found reason to put more emphasis on the shunting

The injection of morphine was followed by a slight increase in PCO_2 and an even smaller further decrease in PO_2 This indicates that there is a slight tendency to depression of the alveolar ventilation and that, if there is any change in the alveolar/arterial gas exchange at all it seems to be improvement rather than impairment This may be correlated with on the one hand the central respiratory depressant effect of morphine and on the other possible hemodynamic effects of morphine

The patients with pulmonary congestion showed more pronounced blood gas abnormalities than those without, both before and after morphine The injection of morphine was followed by a clear increase in PCO_2 but in the patient with the most serious disturbances of the alveolar/arterial gas exchange the morphine induced decrease in the alveolar ventilation was not associated with any further impairment of the ventilation/perfusion disturbances

As regards the oxygen transport to the tissues it is seen that morphine caused a definite decrease in the arterial SO_2 in the patients with pulmonary congestion whereas in the other patients there was only a slight tendency to SO_2 decrease The SO_2 decrease was due partly to the fall in PO_2 and partly to the fall in pH None of the patients studied showed any change in the base excess (7) after the injection of morphine The slight decrease in pH is therefore above all a consequence of the increase in PCO_2 and cannot be ascribed as might have been suspected to a lactic acidosis due to arterial hypoxemia and/or reduced peripheral perfusion

Thus in these patients there was no evidence that the injection of morphine was followed by gross insufficiency of tissue oxygenation However the possibility cannot be excluded that the lowered hemoglobin oxygen saturation before and especially after the injection of morphine may have had an unfavourable effect on the injured myocardium particularly in the patients with pulmonary congestion

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THE EFFECT OF MORPHINE ON BLOOD PRESSURE AND CARDIAC OUTPUT IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Abstract A double blind investigation of the hypotensive effect of morphine in patients with uncomplicated myocardial infarction has been performed in 20 patients. Ten were given an intramuscular injection of 1.5 cg morphine sulphate and ten an injection of a placebo Blood pressure was recorded with the patient lying flat in bed, head up in an armchair position. A slight tendency to development of orthostatic hypotension was observed after the injection of morphine Cardiac output was determined in five patients with uncomplicated myocardial infarction after the i.v injection of 0 75 cg morphine sulphate No marked fall in cardiac output was observed

Morphine is one of the drugs most commonly used in the treatment of patients with acute myo cardial infarction Although it has been widely used for many years the circulatory effect of morphine has not been much studied in man From the few investigations performed it appears as first observed by Drew et al in 1946 (2) that morphine enhances the tendency to develop orthostatic hypotension in the normal subject

Investigations in patients with acute myocardial infarction are even more scarce Thomas et al (6) have demonstrated that severe hypotension may follow the intravenous administration of morphine in patients with acute myocardial in farction This observation has led to investigations of the circulatory effects of alternative analgetics In one investigation a fall in blood pressure was observed after the injection of pethidine (5) while heroine was found by the same investigators to have little effect on the cardiovascular system (4)

We feel that further investigations with regard to the hypotensive effects of morphine in patients with acute myocardial infarction are warranted. The results of such an investigation performed in Medical Department VIII Ullevaal Hospital on patients with uncomplicated acute myocardial in farction are given in this report

MATERIAL

Patients with uncomplicated acute myocardial infarction were examined within 48 hours after the onset of symptoms. Excluded were patients with congestive failure or cardiogenic shock, patients who were not able to lie flat in bed and patients who did not tolerate an armchair position in bed for 15 min All the patients had regular sinus rhythm.

METHODS

Investigations were not started until 6 hours had passed since the last injection of an analgetic. Oxygen was given by face mask at a flow of 5 l per min.

In the first investigation the orthostatic effect of morphine was investigated. Blood pressure was measured by the conventional technique in the right arm to the nearest 2 mm Hg

The investigation was double blind. Ten patients received an injection of 1 5 cg morphine sulphate intra muscularly and another ten patients were given an in jection of a placebo

Blood pressure and pulse rate were first recorded with the patients lying flat in bed. The bed was then tilted until the patients were sitting in an armchair position and the blood pressure and pulse rate were subsequently recorded every 3 min for 15 min. The same procedure was repeated after an intramuscular injection of mor phine and placebo respecuvely The time schedule for the investigation appears in Figs 1–3

In the second investigation the intra arterial pressure was measured with a Statham pressure transducer P 23 GB. Cardiac ouptut was determined by dye dilution technique as previously described (3) After an initial rest of 30 min the patients were tilted 20 head up and cardiac output and blood pressure were registered. Morphine sulphate 0 75 cg was then slowly injected intravenously

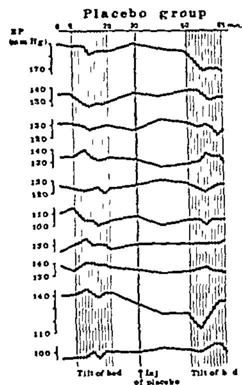


Fig 1 Postural changes in systolic blood pressure before and after placebo

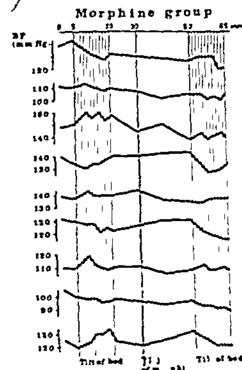


Fig 2 Postural changes in systolic blood pressure before and after morphine

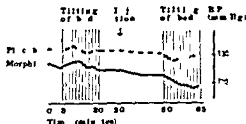


Fig 3 Average blood pressures in morphine and placebo group

during 5 min. Subsequent recordings of cardiac output intra arterial blood pressure and pulse rate were performed 10 and 25 min after the start of the injection.

RESULTS

Orthostatic hypotension after morphine

The results from the double blind investigation with regard to the orthostatic hypotension after morphine appear from Figs 1–3 The systolic blood pressure recorded in the patients in the placebo group is shown in Fig 1 No marked orthostatic hypotension was seen in any of the patients The systolic blood pressure in the patients who were given morphine is illustrated in Fig 2 No pronounced hypotension following the injection of morphine was registered Neither bradycardia nor tachycardia was seen after the injection of morphine None of the patients suffered nausea or vomiting Average systolic blood pressures in the two patient groups are compared in Fig 3 The average systolic blood pressure is higher in the placebo than in the morphine group In both groups the systolic blood pressure fell during the investigation in the placebo group by an average of 4 mm Hg, in the morphine group by an average of 8 mm Hg.

The difference between the two groups with regard to orthostatic hypotension before and after morphine and placebo respectively was not statistically significant

One patient in the control group had a labile hypertension with a marked fall in blood , during the second tilting of the bed If this patient is excluded no orthostatic fall in blood pressure was observed in the placebo group the difference between the two groups with regard to orthostatic fall in blood pressure after the injection of morphine and placebo would then statistically significant (0 02 P 0 01)

Table I *Cardiac output before and after injection of morphine*

In each patient two or three measurements were taken be fore morphineinjection one 10 min and one 25 min after the injection

Pat. no	CO before morphine (l min)	CO 10 min after morphine (l min)	CO 25 min after morphine (l min)
1	4 6 4 3	5 2	4 3
2	5 [illegible] 5 7 5 4	5 0	5 2
3	4 7 4 2	5 6	4 4
4	4 3 4 5 5 0	4 7	4 7
5	7 9 5 9	5 8	5 0

Cardiac output after injection of morphine

The results of the measurements of cardiac out put are shown in Table I No marked fall in cardiac output was observed in any of the pa tients In one patient the systolic and diastolic blood pressure fell by 10 mm Hg following the injection of morphine whereas the cardiac output remained unaltered In the other four patients no change in blood pressure was registered

DISCUSSION

The present investigation did not demonstrate any marked hypotensive effect of morphine in patients with uncomplicated acute myocardial infarction A slight tendency to the development of orthostatic hypotension was however demonstrated This may be of clinical importance during the transport of patients with acute myocardial infarction If hypotension occurs as a result of the injection of morphine the foot end of the bed should be raised as advocated by Thomas et al (6) During transport care must be taken not to carry the patient in a head up position after he has been given morphine

Nausea and vomiting are frequently seen in patients with acute myocardial infarction and may usually be regarded as manifestations of the dis ease Bradycardia and hypotension may be caused by the nausea However morphine in itself may provoke such symptoms although it was not ob served in the present investigation Bjerkelund et al (1) have shown that perphenazin (Trilafon) provides protection against nausea and vomiting in patients with acute myocardial infarction We therefore routinely give perphenazin to patients who suffer nausea or vomiting after the injection of morphine

CONCLUSION

From the present study of the circulatory effects of morphine in patients with uncomplicated acute myocardial infarction we are led to the conclusion that there is no strong justification for the advice that morphine should be substituted by other anal getics in the treatment of these patients

However one must be prepared to encounter the occasional hypotensive reaction caused by morphine It can be controlled by elevating the patient s legs

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CORONARY OCCLUSION TREATED WITH SMALL DOSES OF HEPARIN

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Abstract In common antithrombotic therapy the dosage of heparin is kept at a certain level based on prolongation of in vitro coagulation time However considerably lower doses have been reported to have effect in long term treatment of coronary heart disease In order to investigate the effect of similar low doses in patients with acute coronary occlusion a double blind trial was undertaken, in which the death rates of patients treated with heparin for 24 days at a dosage of 10 000 units subcutaneously twice daily for the first 16 days and subsequently once daily was compared with a placebo-treated group

Thirty two per cent of 103 heparin treated patients died compared with 41 3 of 109 in the placebo group This difference is not statistically significant Thrombo-embolic complications were however significantly less frequent in the treated group and it is concluded that the therapeutic experiment should be continued

Antithrombotic treatment of coronary occlusion by heparin is usually based upon a considerable reduction of the blood s ability to coagulate in vitro and the doses needed vary from 5000 to 15 000 units given intravenously at intervals from 4 to 8 hours When subcutaneous injections are preferred 25 000 units twice daily are considered sufficient

Engelberg et al (3) in 1956 demonstrated that long term treatment of coronary heart disease with small doses of heparin—200 mg subcutaneously twice weekly—had a striking effect on the incidence of new coronary infarctions and sudden heart deaths These observations were confirmed by Bottiger et al (2) in 1965 who made use of the same double blind technique

In view of differences of opinion concerning the value of anticoagulant therapy in acute coronary occlusion it was considered important to investigate whether the course of the acute disease could be influenced by heparin in similar small doses

The oportunity for undertaking such a study presented itself in Denmark in 1961 After the publication by Hilden et al (4) of the unsatisfactory results of anticoagulant therapy it was no longer thought unethical to withhold this treatment from patients

METHODS

From November 1961 until June 1968 all patients with coronary occlusion under the age of 75 admitted to the Medical Department of the County Hospital Hobro were given either 10 000 units of heparin subcutaneously twice daily for 16 days followed by 10 000 units daily for 8 days—or corresponding volumes of placebo

The trial was carried out according to the double blind principle Heparin and placebo preparations were supplied by Leo Pharmaceutical Products Copenhagen in uniform packages each containing four capped vials with colourless fluid, half of which were solutions of heparin 10 000 units per ml. Each vial had a four-digit number referring to a secret code that was kept by a reliable non medical assistant. At the beginning of the treatment a package was taken at random from a minor depot the vials numbers were noted in the record and the patient s name was written on the package

The diagnosis was made as soon as possible after the admission based upon the clinical details and ECG using 2 chest leads According to the following course of the disease repeated ECG temperature reaction ESR and G-O transaminase the diagnosis was confirmed or rejected Autopsy was performed in 62 cases of death (80)

During part of the period coagulation time was determined in the patients at intervals of one day one week and three weeks after the beginning of the treatment Venous blood was taken in a clean and dry glass tube which was slightly tilted every minute and watched until the beginning of visible coagulation The results were kept secret in the laboratory and only considered for statistical analysis at the end of the trial

The treatment received by the individual patients was revealed at intervals when the series of patients had finished treatment, the issue was known and all prognostic criteria and complications had been recorded

Table I *Age distribution*

Age	Heparin group No	Placebo group No
35–44	3	4
45–54	14	15
55–64	35	41
65 74	51	49

Table II *Distribution of risk factors*

	Heparin ( )	Placebo ( )
Previous coronary occlusion	17 5	23 0
Diabetes mellitus	9 7	6 4
Shock	5 8	5 5
Pulmonary oedema	13 6	8 3
Conduction disturbances	15 5	20 2
Congestive heart failure	20 4	23 8

Table III *Death rate*

Risk group	Heparin group Treated no	Heparin group Dead (no)	Heparin group Dead ( )	Placebo group Treated no	Placebo group Dead (no)	Placebo group Dead (%)	p
I	5[illegible]	5	9 6	55	11	[illegible]0 0	0 13
II	51	8	54 9	54	34	63 0	
Total	103	33	32 0	109	45	41 3	0 16

The patients were mobilized according to their individual clinical state by means of a 3-step graduation of bed rest after which they were taken out of bed The average time spent in bed before getting up was 11.3 days in the survivors

In other respects, treatment of the disease followed the traditional pattern and did not change much during the nearly seven year experimental period

RESULTS

The diagnosis of coronary occlusion was made 263 times during the period However in 25 cases the diagnosis eventually turned out to be incorrect In nine of these patients the correction was made at the autopsy table Thirteen patients had to be excluded as they died before therapy could be applied five of these were diagnosed at autopsy Patients who died less than one hour after the first injection are also included in this group and not considered in the study Thirteen patients were thought unfit for the trial for various reasons so the material used in the study was taken from 212 cases

80 2 % of the patients were males corresponding percentages for the heparin and placebo groups were 78 6 and 81 7 The age distribution is shown in Table I The average ages were 62 6 and 62 9 years in the heparin and placebo groups respectively

The distribution of common risk factors is shown in Table II Diabetes mellitus and pulmonary oedema at admission were seen more frequently in patients treated with heparin while previous coronary occlusion congestive heart failure and arrhythmias at the time of admission were more frequent in the placebo group

The result of the therapy is shown in Table III In risk group II one or more of the criteria mentioned in Table II were present in the patients None of the differences between heparin and placebo groups in Table III are statistically significant

Thrombo-embolic complications were recorded in 14 patients treated with heparin (13 6 %) and in 28 patients in the placebo group (25 7 %) the difference being significant ($p = 0.024$) In placebo patients these complications may have been more serious since 18 of them died compared with six deaths in the heparin group The various types of complications recorded are shown in Table IV

Table IV *Thrombo-embolic complications*

	Number of cases Heparin	Number of cases Placebo
Reinfarction	4	4
Phlebitis	5	10
Pulmonary emboli		6
Systemic emboli	3	9
Parietal thrombi found at autopsy	5	14
Parietal thrombi clinically diagnosed	0	1

Table V *Coagulation time*

	No	1 day after admission Mean (sec)	1 day after admission S D (sec)	[illegible] weeks after admission Mean [illegible] ( )	S D ( )
Heparin	38	[illegible]1 6	11 4	5 8	6 8
Placebo	[illegible]8	209 9	1[illegible]6	11 8	[illegible]

A few haemorrhagic complications were seen in both groups none of them was severe Coagulation times are shown in Table V The differences between heparin and placebo treated patients are slight and not statistically significant

DISCUSSION

According to prevailing concepts antithrombotic heparin therapy requires an augmentation of the coagulation time to 2–3 times the normal value Some authors claim coagulation times of 20–30 min

An analysis of the rationale of this procedure reveals some facts and some assumptions Evidently determination of coagulation time is a practical method of measuring a single biological effect of heparin The result depends in a predictable way on dose method of application and duration after the injection

It is also a fact that by injections especially using the intravenous route a considerable variation of concentration is obtained so that the effect at certain times must be a good deal greater than the therapeutic minimum

It is assumed firstly that the antithrombotic effect of heparin is parallel to its ability to impede the coagulation of blood in vitro and secondly that the very strong effect sometimes met in injection therapy is on the whole harmless

However the results of heparin therapy according to these principles did not show any advantage when compared with warfarin treatment (1) therefore it seemed justifiable to investigate a possible antithrombotic effect of heparin at a dosage lower than that previously used

In the present series anticoagulation in the usual sense has not been intended nor has it been obtained as judged by the coagulation times found Serious haemorrhagic complications were not expected and not observed This suggests an advantage over common anticoagulation measures in which the efficacy of the therapy may be partially counterbalanced by its complications

Studies on the effect of anticoagulation in coronary occlusion frequently do not include patients who died less than 48 hours after the beginning of the treatment even if this period is covered by heparin However a great part of the fatal issues are seen during this first period and it is obvious that future investigations of antithrombotic therapy should deal also with this critical period

No definite conclusion can be made from this therapeutic experiment Although the patients treated with heparin had a lower mortality rate *the number of patients studied was too small to* demonstrate that this difference in mortality did not occur by chance The difference in thromboembolic complications may be a real effect of heparin in low dosage and suggests that the investigation should be continued

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PQ INTERVAL DEPENDING ON POSTURE

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Abstract Three healthy persons with prolonged PQ duration in supine position are presented The PQ is normal in standing position. From these cases and from the literature it is concluded that a prolonged PQ-interval in itself is no indication of heart disease and that in such cases a standing ECG should always be taken.

The PQ interval in the electrocardiograms is normally within the range of 0 12 to 0 22 sec The duration is to some extent correlated to the heart rate Abnormal PQ intervals are seen in patients with acute rheumatic fever in acute myocardial infarction and in digitalis intoxication

As abnormal PQ duration is most often seen in heart disease we find it is important to stress that the PQ is seen to change significantly with posture in completely healthy persons

New documentation of this fact is given by the history of three patients in whom the PQ interval was dependent on posture A fourth case with arteriosclerotic heart disease with prolonged PQ not changing with posture is presented for comparison

CASE REPORTS

Patient 1

A 0-year-old male who was first seen in our department at the age of 14 in 1961

He had no heart complaints

At auscultation a systolic murmur was heard at the left sternal border The X ray of the chest was normal The ECG showed a prolonged PQ-interval.

A right heart catheterization was done showing normal pressures and no intracardial shunts

The patient was not seen until 1968 when he had to join the army He was still without heart complaints X ray of the chest was still normal

At auscultation a systolic murmur grade 1 was heard at the left sternal border It was of vibratory type

The ECG was normal apart from a prolonged PQ-interval of 0.30 sec (Fig. 1) Standing ECG showed a PQ of 0 18 (Fig 2) When the patient was asked to lie down again the PQ after two min delay was again prolonged (0 30 sec) One mg atropine was given intravenously Shortly after this the PQ fell to 0 18 sec The effect subsided after some hours.

During exercise the PQ fell to 0 18 sec

Conclusion

No organic heart disease PQ-interval changes with posture.

Patient 2

A 39 year-old woman, who was admitted with fever The diagnosis was pyelitis

As the ECG showed prolonged and changing PQ interval, rheumatic fever and myocarditis were suspected The examinations could not substantiate this suspicion. During treatment with antibiotics, the fever subsided

The patient was then seen in our department. She had never fainted and had never had cardiac complaints

At auscultation no murmurs were heard Blood pressure was 100/70 The ECG is seen in Fig 3 The PQ-interval was 0 ?8 sec Standing ECG is shown in Fig 4 PQ was 0 18 sec The patient was asked to lie down again and after two min the PQ was 0 34 sec

X ray of the chest was normal AST 65-100 (normally below 200) ASH below .000 (normally below 4000) L.E. cells negative

Conclusion

No organic heart disease PQ-interval changed with posture

Patient 3

A .1 year-old male who was admitted for tonsillectomy There was no family history of congenital heart disease He had never had rheumatic fever or tuberculosis

Since his early childhood he had had tonsillitis several times.

He had never had heart complaints and was a top-trained athlete During six months he had had intermittent joint pains, but there had never been swelling of any joint.

He had never fainted

At auscultation no murmur was heard. The second heart sound was split in the pulmonary area

The X ray of the chest was normal

The ECG (Fig. 5) showed right bundle branch block. There was wandering pacemaker and nodal rhythm. Stand

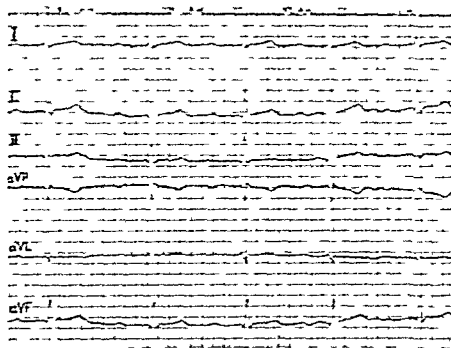


Fig. 1. Patient no. 1. ECG in supine position. PQ 0.3? sec. Heart rate 75. Paper speed 50 mm/sec.

ing ECG is shown in Fig. 6. The PQ was now normal. During and after exercise there was also normal sinus rhythm. The heart rate was 168.

ESR 5 mm in one hour. Hb 14.5 g%. AST and ASH normal.

No signs of rheumatic fever or rheumatic heart disease. Right bundle branch block and wandering pacemaker were found. In standing position the PQ was normal. His heart was considered to be normal.

Patient 4

A 72-year-old male with angina pectoris since 19?1. During the last six years he had been subject to fainting fits. He complained of dyspnoea during exertion.

In 1966 he got a left-sided pa esis.

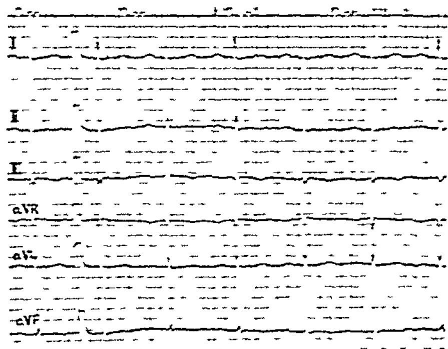


Fig. Patient no. 1. ECG. PQ 0.38 sec. Heart rate 9?. Paper speed sec.

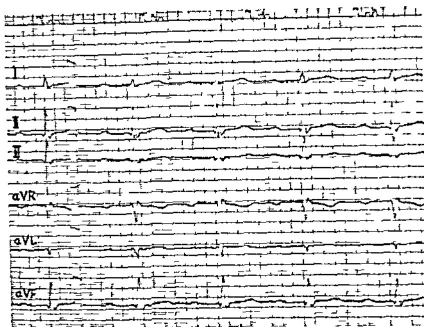


Fig 3 Patient no 2. ECG in supine position. PQ 0 28 sec. Heart rate 70 Paper speed 50 mm/sec.

He was now admitted because an A V block had been found

At auscultation no murmurs were heard. Blood pressure 150/60

No arterial pulsations could be felt in the feet.

The X ray showed an enlarged heart.

The ECG showed a left bundle branch block and a PQ-interval of 0.28 sec

The patient was not on digitalis treatment and there was no evidence of recent myocardial infarction.

Standing ECG and ECG after atropine still showed a PQ of 0..8 sec

Conclusion

Arteriosclerotic heart disease The prolonged PQ was not normalized in the standing position.

The faintings could have been Stokes-Adams attacks.

DISCUSSION

Atrio-ventricular block of first second and third degree may be periodic or constant All three types are seen in congenital heart disease either as a part of it or acquired, superimposed upon the deformity The most common congenital defect with heart block is atrial septal defect (5) Block is also seen in digitalis intoxication lues rheumatic fever diphtheria chorea and in virus infections The block in these cases is often of first or second degree but total A V block may be seen and was described in a 23 year-old soldier with German measles (3) The block disappeared when the infection subsided

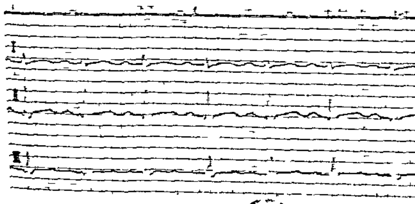


Fig 4 Patient no 2. Standing ECG PQ 0 18 ... rate 100 Paper ... mm sec

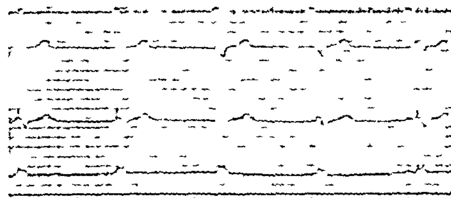


Fig. 5. Patient no. 3. ECG in supine position. Right bundle branch block. Nodal rhythm. Heart rate 60. Paper speed 50 mm/sec.

In acute myocardial infarction A-V block, incomplete or complete, transient or constant, is frequently seen.

It is characteristic that, although the block may change during disease, it has never been described as dependent upon the position of the patient.

Total A-V block does not change with posture, not even in congenital block without other heart abnormality. In all these cases faintings may be seen.

The question now arises whether an abnormal, especially prolonged, PQ-interval is seen in healthy persons and whether this PQ could be dependent upon the posture of the patient. From the foregoing remarks, this should be suspected only in A-V block of first and second degree. As seen from patient no. 3 postural normalisation of the PQ may be expected in wandering pacemaker and may also be found in other disturbances of the A-V conduction.

From the literature is known that A-V block is found in healthy persons. In a Finnish series of 650 athletes (2) a PQ interval of 0.22 to 0.40 sec was found in 3 cases. This abnormal data on changed even during short time intervals but never became normal. On the other hand three of these athletes had a normal PQ when they were seen 20 years after they had given up athletics.

What posture means to the PQ has also been earlier described. Twenty normal men, members of the Royal Air Force, showed a prolonged PQ in supine position (4), while sitting or standing ECG showed a normal PQ.

In this series the heart rate had no significant influence on the PQ interval. The men were examined on tilt tables and the PQ was normal in a position 50 degrees from the horizontal.

A follow-up study was performed on these students several years after the first examination. Th

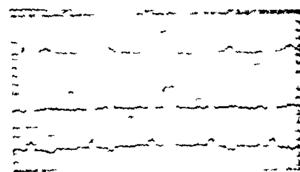


Fig. 6. Patient no. 3. Standing ECG. PQ 0.1 sec. Heart rate 80. Paper speed 50 mm/sec.

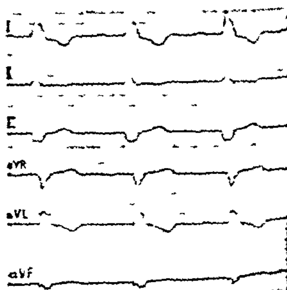


Fig. 7. Patient no. 4. S... ECG. PQ 0. sec. Heart rate 60. Left bundle branch block. Paper speed ... mm/sec.

PQ was still prolonged and still changing with posture No signs of organic heart disease were found

Three healthy men were seen by Holmes and Weill (1) who found a prolonged PQ in supine position becoming normal in standing position They found no correlation to heart rate

The conclusion is that a prolonged PQ interval or otherwise abnormal PQ is not in itself an indication of organic heart disease

If the history and all other examinations are normal a normal standing ECG speaks against organic heart disease

The reason for a prolonged PQ is not known In our patients the heart rate was higher in standing position and as atropine made the PQ normal a hi h vagal tone might be the explanation in these cases Physical training may give a prolonged PQ and this could also be due to vagal tone but in many cases no definite explanation can be given

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GASTRIC SECRETION OF ACID AND INTRINSIC FACTOR IN PATIENTS WITH HYPER AND HYPOTHYROIDISM

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From Medical Department II Sahlgren s Hospital University of Goteborg Goteborg Sweden

Abstract Fifteen consecutive thyroid disease patients, ten with hyperthyroidism and five with hypothyroidism have been investigated as far as gastric secretion was concerned Gastric secretion of acid and intrinsic factor was studied in the patients both basally and with the augmented histamine test. In eight patients with hyperthyroidism and all patients with hypothyroidism the gastric secretion of acid after histamin stimulation was significantly lowered The secretion of intrinsic factor correlated with the secretion of acid and was significantly depressed in four patients with hyperthyroidism and three with hypothyroidism Further studies showed pernicious anemia in two patients with hypothyroidism.

Disturbances of gastric secretion as related to thyroid gland dysfunction have been noted for many years After the augmented histamine test normal or low acid output has been registered both in hyperthyroidism and hypothyroidism (3 4 11) An association with pernicious anemia has been described in both groups

In an earlier publication we have noticed a high incidence of achlorhydria hypochlorhydria and atrophic gastritis in patients with hyper and hypothyroidism (7) The present paper deals with the findings on gastric secretion of acid and intrinsic factor in these two conditions

MATERIAL

Fifteen consecutive thyroid disease patients four men and 11 women, entering either a medical or surgical ward were investigated (Table I) The mean age of the ten patients studied with hyperthyroidism was 42 years and of the five patients with hypothyroidism 59 years The patients had a typical clinical history of hyper or hypothyroidism. In all cases except nos 3 4 and 10 PBI was taken and the highest value recorded (Table I) In these cases however BMR and/or RAI were taken In cases 3 and 10 subtotal thyroidectomy was performed Case 3 was later treated with radioactive iodine for recurrency Case 4 was successfully treated with thyroxin blocking agents only Five patients were euthyroid at the secretory test. Cases 2 and 9 were studied pre-operatively after having been euthyroid for 2-4 weeks. In cases 3 4 and 7 the gastric secretory test was performed 3 10 and 3 years respectively after they were made euthyroid

METHODS

Gastric secretory studies were performed in the morning after at least 12 hours fasting. A nasogastric tube of 3 mm internal diameter was passed into the stomach The patient was sitting in a semi-upright position. Continuous draining of the stomach was carried out by suction at subatmospheric pressure of 50 mm Hg Extra suction and injection of air was applied by means of a syringe After removal of the residual secretion from the stomach a 60 min collection of basal secretion was made Midway through this period 100 mg antazoline was given by intramuscular injection At the end of the basal secretion, histamine HCl was given in doses giving maximal acid output (9) The volume of the gastric juice collected was measured and samples taken from each specimen for determination of HCl and intrinsic factor

Hydrogen ion activity was determined in an automatic pH meter (Radiometer Copenhagen) Gastric juice was titrated with 1/10 NaOH until pH 7 0 Results are given as mEqH /h.

The samples for intrinsic factor assay were neutralized with 10 *N* NaOH to pH 7 5 centrifuged to remove mucus and stored at − 23 C before assay Intrinsic factor activity was determined as described by Ardeman and Chanarin (1) using Co vitamin B as radioactive isotope Intrinsic factor was expressed as the difference between total vitamin B binding capacity and the vitamin B_{12} binding capacity after complete blockage of intrinsic factor with excess of antibodies against intrinsic factor One unit of intrinsic factor was defined as the specific intrinsic factor binding of 1 ng vitamin B_{12}.

Schilling test

The Schilling test was carried out as described by Grasbeck et al (8) in some of the patients with achlorhydria or low acid output after histamine stimulation.

Table I *Clinical and laboratory data on 15 patients studied with hyper or hypothyroidism*

Pat. no	Age	Sex	Diagnosis	PBI (μg %)	Thyroid function at the secretory study	Remarks
1	60	♂	Hyperthyr	11 7	Hyperthyroid	
2	45	♀	Hyperthyr	9 9	Euthyroid	
3	56	♀	Hyperthyr	—	Euthyroid	BMR +56 RAI 7 h 64 Schilling test 26%
4	55	♂	Hyperthyr	—	Euthyroid	BMR +35% Schilling test 17
5	59	♀	Hyperthyr	12 1	Hyperthyroid	Serum Vitamin B_{12} 250 pg ml
6	20	♀	Hyperthyr	12 3	Hyperthyroid	
7	54	♀	Hyperthyr	13 8	Euthyroid	
8	26	♀	Hyperthyr	13 0	Hyperthyroid	
9	19	♀	Hyperthyr	9 9	Euthyroid	
10	76	♂	Hyperthyr	—	Hyperthyroid	BMR +35
11	67	♂	Hypothyr	1 8	Hypothyroid	Schilling test without intrinsic factor 4% with intrinsic factor 11
12	52	♀	Hypothyr	0 9	Hypothyroid	
13	64	♀	Hypothyr	2 5	Hypothyroid	
14	56	♀	Hypothyr	2 0	Hypothyroid	Schilling test without intrinsic factor 8%
15	45	♀	Hypothyr	1 9	Hypothyroid	

RESULTS

Gastric secretion of acid

al secretion of acid was significantly lowered patients with both hyper and hypothyroidism compared with controls (Figs 1 and 2) After histamine stimulation the acid output was within normal limits in two patients with hyperthyroidism (Figs 3 and 4) For all other patients the secretion of acid was significantly lowered

Gastric secretion of intrinsic factor

Basal secretion of intrinsic factor was investigated in ten subjects with hyperthyroidism in four patients the intrinsic factor secretion was lower than the lower range for hospital patients (2) (Fig. 5)

In all patients with hypothyroidism the basal secretion of intrinsic factor was low in three subjects < 100 units/h (Fig 6)

After histamine stimulation the secretion of in

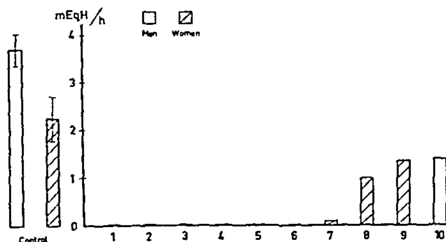


Fig 1 Basal secretion of acid in mEqH /h in ten patients with hyperthyroidism. Mean and S.E. for controls according to Dotevall (6)

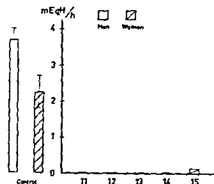
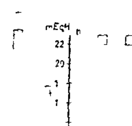


Fig 2 Basal secretion of acid in five patients with h thyroidism. Mean and S.E. for controls



trinsic factor was within normal limits ir f patients with hyperthyroidism (Fig 7) In a other patients the secretion of intrinsic fa tc low (Figs 7 and 8) in four patients with h thyroidism and three patients with hypothyroid m lower than the lower range for hospital patient (2)

DISCUSSION

Our findings of low acid output in patien ith hypothyroidism and hyperthyroidism are in agree ment with those of others In an earlier publi a tion (7) we found a good correlation between

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ga ... y findings in pat e ... Eight of 17 ... had atrophi ... ghly de press d ... ated hista mine te ... thyroidism

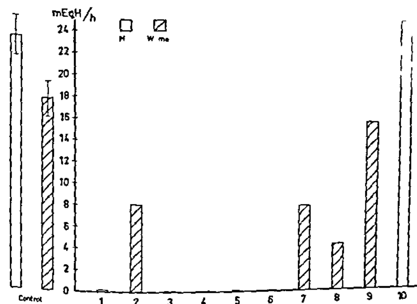


Fig 3 Gastric secretion of acid in mEqH /h after maximal histamine stimulat on in ten pat ents with hyperthyroidism Mean and S.E. for controls according to Dote all (6)

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7	54	♀	Hyperthyr	13 8	Euthyroid	
8	26	♀	Hyperthyr	13 0	Hyperthyroid	
9	19	♀	Hyperthyr	9 9	Euthyroid	
10	26	o	Hyperthyr	—	Hyperthyroid	BMR +35
11	67	♂	Hypothyr	1 8	Hypothyroid	Schilling test without intrinsic factor 4 with intrinsic factor 11 %
12	52	♀	Hypothyr	0 9	Hypothyroid	
13	64	♀	Hypothyr	2.5	Hypothyroid	
14	56	♀	Hypothyr	2 0	Hypothyroid	Schilling test without intrinsic factor 8 %
15	45	♀	Hypothyr	1 9	Hypothyroid	

RESULTS

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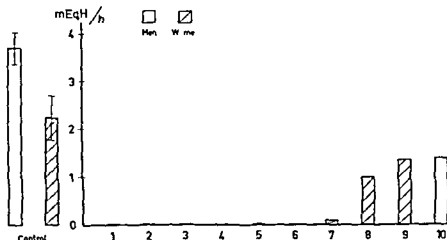


Fig 1 Basal secretion of acid in mEqH /h in ten patients with hyperthyroidism. Mean and S.E. for controls according to Dotevall (6)

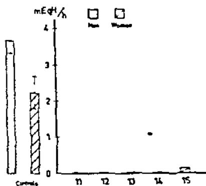


Fig ? Basal secretion of acid in five patients with hypothyroidism. Mean and S.E. for controls.

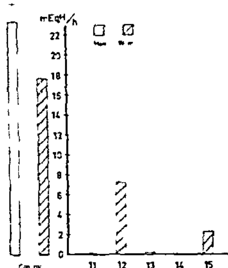


Fig 4 Gastric secretion of acid in mEqH /h after maximal histamine stimulation in five patients with hypothyroidism. Mean and S.E. for controls.

trinsic factor was within normal limits in four patients with hyperthyroidism (Fig 7) In all other patients the secretion of intrinsic factor was low (Figs 7 and 8) in four patients with hyper thyroidism and three patients with hypothyroidism lower than the lower range for hospital patients (2)

DISCUSSION

Our findings of low acid output in patients with hypothyroidism and hyperthyroidism are in agree ment with those of others In an earlier publica tion (7) we found a good correlation between gastric acid secretion and gastric biopsy findings in patients with thyroid gland disease Eight of 17 subjects studied with hyperthyroidism had atrophic gastritis and achlorhydria or highly de pressed acid secretion after the augmented hista mine test. In 15 patients with hypothyroidism

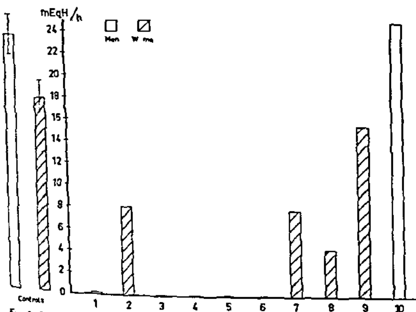


Fig 3 Gastric secretion of acid in mEqH h after maximal histamine stimulation in ten patients with hyperthyro dism. Mean and S.E. *for* controls according to Dotevall (6)

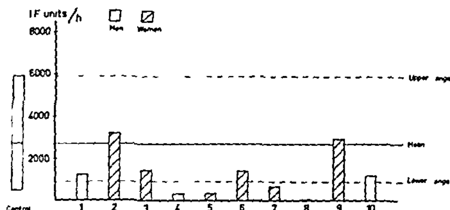


Fig 5 Basal secretion of intrinsic factor in units/h in ten patients with hyperthyroidism Mean ± range for controls according to Ardeman et al. (2)

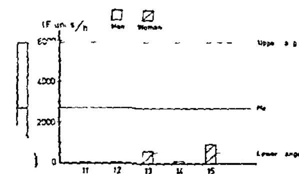


Fig 6 Basal secretion of intrinsic factor in units h in five patients with hypothyroidism. Mean ± range for controls

atrophic gastritis was seen in eight subjects who had achlorhydria or low acid output after the augmented histamine test

As far as hyperthyroidism is concerned a high incidence of circulating antibodies to the gastric mucosa has been found (5) The report from Bock and Witts (3) of the connection between latent pernicious anemia hypochlorhydria and achlorhydria in patients who had recovered from hyperthyroidism is of interest In the present study three of the patients had had their hyperthyroidism some years before the gastric study and were euthyroid at the examination Two of them (nos 3 and 4) had achlorhydria after the augmented histamine test and low values for intrinsic factor 1386 μ/h and 209 μ/h respectively The Schilling test was however normal in both cases All other patients had their hyperthyroidism diagnosed at the time of the study Two patients were made euthyroid by thyroxin blocking drugs before the secretory test. They had a normal acid secretive response after histamine stimulation and the secretion of intrinsic

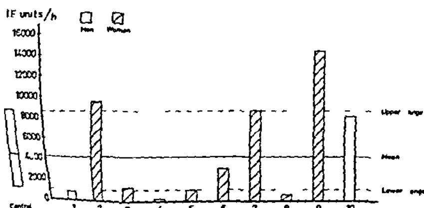


Fig 7 Gastric secretion of intrinsic factor in units h after maximal histamine stimulation in ten patients with hyperthyroidism Mean ± range for controls according to Ardeman et al ( )

Fig 8 Gastric secretion of intrinsic factor in units/h after maximal histamine stimulation in five patients with hypothyroidism Mean ± range for controls

factor was within normal limits Among the five subjects who were thyrotoxic at the study four had achlorhydria or highly depressed acid output after histamine stimulation. Three of the patients had a secretion of intrinsic factor of < 1000 units/h These values are abnormally low according to the data for controls and hospitalized patients presented by Ardeman et al (2) Further studies did not reveal any manifest pernicious anemia

Five patients with hypothyroidism were studied as far as gastric acid secretion was concerned Three of them had achlorhydria or highly depressed acid secretion two had low acid output The secretion of intrinsic factor was < 1000 in three and within normal limits in two patients Further studies showed pernicious anemia in one case (no 11) and the latent pernicious anemia in another (no 14) Our results support the observations of a connection between hypothyroidism and pernicious anemia. As in hyperthyroidism a high incidence of parietal cell cytoplasmic antibodies has b en found in patients with Hashimoto s disease and hypothyroidism (5 10)

CONCLUSION

The material of hyper and hypothyroidism presented here is small but the results confirm earlier findings of a low acid output in these conditions The secretion of intrinsic factor correlates with the secretion of acid and is low in hypochlorhydria and achlorhydria This is in agreement with th clinical association between thyroid gland disease and pernicious anemia

ACKNOWLEDGEMENT

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URINARY EXCRETION OF HYDROXOCOBALAMINE AND CYANOCOBALAMINE AFTER ORAL ADMINISTRATION OF LARGE DOSES

Hans Hedstrand

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Abstract Eight patients with pernicious anaemia in relapse have been treated with oral doses of .000 μg hydroxocobalamine twi e daily Remission was obtained in all patients within four to six weeks Stud es with labelled cobalamines showed that after large oral doses less OH B_{12} than CN B_{12} was excreted in the urine when equal doses were gi en to the same person This difference was small but significant

Hydroxocobalamine (OH B_1) is retained longer in the human body than cyanocobalamine (CN B_1) after parenteral administration (3 5) Intestinal absorption in man of large doses of vitamin B_1 is not intrinsic factor dependent (2) At oral doses between 100 and 1000 μg the mean absorption of OH B_1 and CN B_1 are not significantly different (6) Thus the greater body retention of OH B_1 would give a lower urinary excretion than that of CN B_1 In the present study the urinary excretion after large oral doses of the two forms of the vitamin has been compared

METHODS

Routine haematological methods were used 24-hour urines were collected in bags containing 10 mg of potassium cyanide the volumes measured and frozen aliquots stored until assayed The vitamin B_{12} activity was estimated microbiologically with Euglena gracilis strains according to Hutner et al. (4) Radioactive oral doses were Co^{60} labelled CN B_{12} and Co^{5} labelled OH B_{12} Each tablet contained 1000 μg of the vitamin with a radioactivity of 1 μC The urine was collected 0–24 h and .4–48 h after the oral dose The rad oactivity was measured with a scintillation counter in an arrangement for spectrometric analysis of Co^{60} and Co peaks

RESULTS

The study was planned in four parts In the first part eight patients with pernicious anaemia in relapse were treated with oral doses of 2000 μg of OH B_1 twice daily (Hepagon tablets 1 mg Astra) All patients were monitored by laboratory control The reticulocyte response was measured haemoglobin values together with serum B_1 estimations were determined every second week Remission was obtained in all patients within four to six weeks (Fig 1)

In the second part of the study oral doses of 2000 μg of vitamin B_1 were given twice daily to male patients hospitalized because of uncomplicated myocardial infarction The patients had no signs of haematological hepatic or renal disease The two forms of vitamin B_1 were given for one week each Fig 2 illustrates the variations in the 24 hour urinary excretions No significant differences were found It must be noted that the excretion was very low less than 0 1 % of a given daily dose

In the third part of the study the urinary excretion of the two forms of vitamin B_1 were compared using equal oral doses of Co^{60} labelled CN B_1 and Co labelled OH B_1 The two forms were given simultaneously in doses from 1000 μg to 4000 μg each A flush dose of 2000 μg of non radioactive CN B_1 or OH B_1 was given 2 and 24 h after the oral dose Table I shows that less OH B_1 than CN B_1 was excreted For ten patients receiving 4000 μg daily of each form the mean 24 hour and 48 hour excretion was 4 7 μg and 8 4 g respectively of OH B_1 and 7 2 μg and 11 5 μg of CN B_1 These differences in urinary excretions are highly significant ($0.001 < p < 0.01$)

Finally daily oral doses of 1000 μg Co^{60} labelled CN B_1 or Co labelled OH B_1 were administered on alternate days to two patients

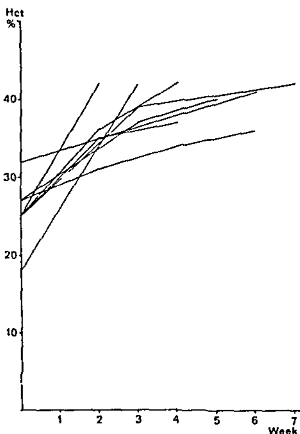


Fig 1 Increase in hematocrit concentration during oral with 2000 μg of OH B twice daily

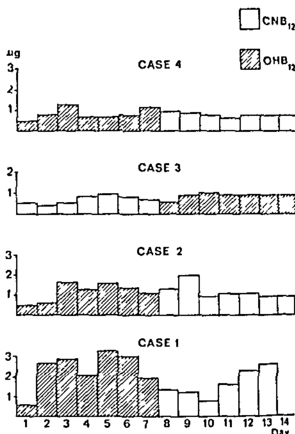


Fig 2 24 hour urinary excretion of vitamin B_{12} following oral doses of 2000 μg twice daily OH B and CN B were given for one week each

Table I *Urinary excretion of vitamin* B_{12} *following identical oral doses of* OH-Co^{57}-B_{12} *and* CN-Co^{60}-B_{12}

dose given as 000 μg of non radioactive OH B_{12} in cases 1 8 and as CN B_{12} in cases 9 13

Case no	Oral dose (μg)		0-24 and 0-48 hour urinary excretion (μg)			
			OH Co^{57}-B_{12}		CN Co^{60}-B_{12}	
	OH-Co^{57} B_{12}	CN-Co^{60}-B_{12}	0-24 hour	0-48 hour	0-24 hour	0-48 hour
1	1000	1000	3 8	4 9	4 2	5 5
2	1000	1000	0 6	0 9	1 4	2 1
3	2000	2000	3 1	4 5	5 2	7 3
4	4000	4000	3 1	5 4	5 5	8 1
5	4000	4000	2 5	4 9	5 8	10 0
6	4000	4000	2 6	5 2	4 0	6 5
7	4000	4000	6 0	10 0	10 3	15 6
8	4000	4000	4 0	10 4	5 9	12 8
9	4000	4000	4 3	5 5	7 5	9 3
10	4000	4000	2 8	6 2	3 0	6 5
11	4000	4000	9	17 9	9 3	12 8
12	4000	4000	8 5	10 2	14 3	16 8
13	4000	4000	4 0	13 0	6 0	16 4

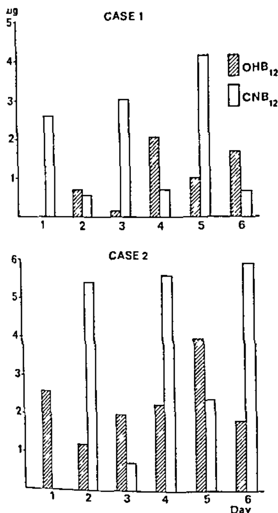


Fig 3 24 hour urinary excretion of vitamin B_{12} following oral dose of 1000 µg OH-Co⁵⁷ B_{12} or CN Co⁶⁰B_{12} CN B_{12} given on days 1 3 and 5 in case 1 and on days 2 4 and 6 in case 2 OH B_{12} given on the other days in each case Daily flush dose containing 500 µg of each vitamin

for 6 days A flush dose containing 500 µg of non radioactive CN B_{12} and 500 µg of OH B_{12} was given 2 h after the oral dose Fig 3 shows the urinary excretion values The mean excretion of each vitamin for 5 days (3 days when tablets were given and the 2 days between these days) in case 1 was 1 1 µg of OH B_{12} and 2 2 µg of CN B_{12} and in case 2 2 3 µg of OH B_{12} and 4 0 µg of CN B_{12}

DISCUSSION

Using urinary excretion studies it has earlier been shown that after parenteral administration OH B_{12} is retained longer in the human body than CN B_{12} (5) This has been confirmed by Boddy et al (3) using whole body monitoring They found a whole body retention of OH B_{12} on the 3rd day of 42 1% after a single injection of 1000 µg The retention of CN B_{12} after the same dose was 13 7% By the 28th day the retention was 32 5% and 10 5% respectively It is also shown that the absorption of large oral doses of the two forms of vitamin B_{12} is not significantly different (6) Berlin et al (2) have shown that this absorption is approx 1 2% of the dose administered within a very wide dose range Abe et al (1) gave oral physiological doses of the two forms to healthy persons The faecal excretion rate did not show a significant difference After a 1 mg flush dose given 2 h after the oral dose the 24 hour urinary excretion of OH B_{12} was significantly lower than that of CN B_{12} or 9 0 ± 1 9 and 26 7 ± 4 8 respectively of the given dose

In the present study the urinary excretion after large oral doses of the two forms of vitamin B_{12} was compared Regardless of dose more CN B_{12} than OH B_{12} was excreted 1000 µg is the recommended maintenance dose of CN B_{12} in oral treatment of pernicious anaemia (2) This study has shown that OH B_{12} is retained significantly better in the human body than CN B_{12} even after large oral doses However the difference is probably of little clinical importance and in the oral treatment of patients with pernicious anaemia the same doses of OH B_{12} as of CN B_{12} are recommended

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EFFECT OF PARA AMINOSALICYLIC ACID ON SERUM LIPIDS

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Abstract The effect of PAS on serum lipids has been studied in a sample of 15 outpatients whose da ly dietary regimes were not controlled PAS was found to reduce serum cholesterol phospholipid and total lipid values, a finding consistent with results reported earlier Apparently owing to the freer dietary cond t ons, the falls in lip d values in the pres nt sample were somewhat lower than those reported earlier Normal triglyc ride values showed no significant fall but two patients with increased triglycerides showed considerable reductions Further stud es into the effect of PAS on triglycerides are obviously worthwhile

A number of drugs have been used prophylactically against premature atherosclerosis but some of them have proved toxic to the human organism while others may have certain adverse side effects e g the thyroid preparations and female sex hormones No completely satisfactory lipid reducing agent has been developed as yet

The effect of PAS on serum lipids is less known As early as 1955 Riska in his studies of Finnish tuberculosis sanatorium patients found that PAS definitely reduced serum cholesterol (10) This finding was confirmed by Tygstrup et al in 1961 from a sample of 24 non tuberculous patients with hypercholesteraemia (13) whose serum cholesterol fell by 30 A detailed study was made by Kerstell and Svanborg (8) in 1966 In addition to cholesterol they included in their study the other serum lipids viz phospholipids triglycerides and total lipids Their sample comprised seven patients and a remarkable fall was produced in all lipid fractions o her than triglycerides which however were normal at the outset

The purpose of the present study based on the above results was to make a supplementary examination of the action of PAS on serum cholesterol phospholipids triglycerides and total lipids in outpatients attending the Department of Medicine University of Oulu The sample consisted of selected patients whose serum cholesterol had already been determined for some reason and been discovered to be above the normal

The sample comprised nine women and six men Their cholesterol values ranged from 280 to 570 mg/100 ml Their dietary regimes and customary ways of life were disregarded but they reported having b en on low fat diets according to instructions received earlier

The study was started by determining (1) cholesterol (2) phospholipids (3) triglycerides and (4) total lipids

The patients then took PAS in the form of sodium salt for exactly one month 12 g per day and a re determination of the same lipids was carried out

METHODS

Serum cholesterol was determined according to Abel et al (1) phospholipids accord ng to van Handel and Zilversmith (5) triglyc rides according to Hawk et al (6) and total lipid according to Henry s method (7)

The normal values accord ng to these methods are

cholesterol 170–270 mg 100 ml
phosphol pids 1.5–3.5 mg 100 ml
triglycerides 60–1 0 mg 100 ml
total lipids 450–1000 mg 100 ml

RESULTS

The results are given in Figs 1 and 2 The lowered percentages were

cholesterol 17 5 %
phospholipids 15 4 %
triglycerides 20 0
total lipids 18 8

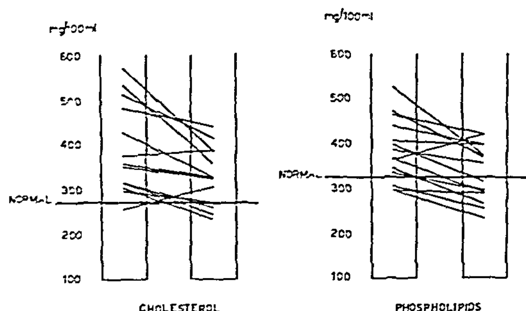


Fig 1 The effect of PAS on serum of cholesterol and phospholipids

The results agree with those reported from earlier studies even though the falls were less pronounced The reason may be that the earlier studies had been carried out under controlled conditions in hospitals, keeping a rigorously fixed diet.

In two cases, with initial triglyceride values of 588 and 319 mg 100 ml the falls were 22 1 and 47 % respectively (cases 3 and 7) It is therefore possible that PAS also reduces increased triglyceride levels a finding to encourage further studies of PAS on hypertriglyceridaemia In triglyceride

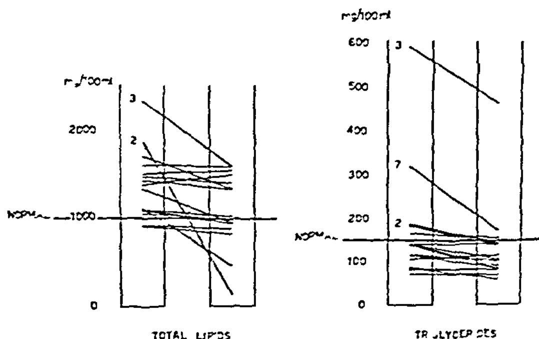


Fig 2 The effect of PAS on serum level of total lipids and triglycerides

series the distribution of the falls was so wide that the result in this study is not statistically significant

DISCUSSION

The mechanism by which PAS affects serum lipids is not known with certainty The assumption is that the steatorrhoea produced by PAS is the effective factor but Tygstrup et al (13) found the amount of steatorrhoea to be too low to account for such considerable reductions in serum lipids (13) Moreover in studies in vitro PAS did not reduce the cholesterol synthesis by the liver although this of course does not exclude a possibility of reduction in vivo The effect of PAS on peripheral fat tissues is unknown The salicylic acid chemically close to PAS reduces free fatty acids in the serum of both normal and diabetic subjects This may result from reduced lipolysis (3) Whether or not PAS has a similar effect is unknown Furthermore salicylic acid and its derivatives have been found to increase the turnover of thyroxin in the organism (14) whereas PAS has sometimes been found to produce hypothyreosis known to be associated with hyperlipaemia (2 9)

It may be pointed out that in diabetes PAS has been found to reduce increased lipids (8 24) It has even been reported in the light of four case reports that the wax like exudates in diabetic retinopathy decreased or disappeared in the course of PAS therapy (4)

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Acta med scand Vol 186 pp 543–547 1969

MECHANICAL HEMOLYSIS IN AORTIC VALVULAR DISEASE AND AORTIC BALL-VALVF PROSTHESIS

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Abst act Using serum haptoglobin concentration as an index, the frequency of hemolysis has been determined in 110 consecutive non selected patients with aortic valvular lesions admitted to Medical Department B Rikshospitalet, during one year from February 1968 Marked lowering or absence of serum haptoglobin was seen in 11 of 65 patients with aortic valvular disease hemolysis being about twice as frequent in aortic in sufficiency as in aortic stenosis Values indicating hemolysis were observed in 37 of 45 patients with a Starr Edwards aortic ball valve prosthesis In most cases hemolysis was slight, no case with clinically important hemolytic anemia was seen. Marked lowering of the serum haptoglobin concentration seems to be the most *sensitive* qualitative indicator of hemolysis the serum lactic de hydrogenase activity probably giving additional informa tion about the rate of hemolysis

Hemolysis due to mechanical trauma of red blood cells was previously seldom observed After the introduction of open heart surgery however this problem has gained considerable interest (23) The early cases of hemolytic anemia following heart surgery were seen in patients operated for septum defects (12 25 29) and following plastic operations on the aortic and mitral valves (28 30) Usually the hemolysis is moderate but fatal cases of hemolytic anemia are seen in patients with foreign material inserted permanently in the heart (17)

As early as 1956 Stohlman et al (27) showed that insertion of ball valve prosthesis in the aorta of dogs invariably caused hemolysis and in 1964 Dameshek (5) observed hemolytic anemia in an unoperated patient with aortic valvular disease During the last years numerous reports concerning hemolysis in aortic valvular disease and aortic valve prosthesis have been published (1 2 6 10 26 32 33) though most series are rather small and the data observed differ considerably (4 7)

To get more precise information about the frequency and degree of the intravascular hemolysis in such patients we decided to study a larger group of non selected consecutive patients suffer ing from different types of aortic valvular lesions and a reasonable number of patients after the insertion of a Starr Edwards aortic ball valve prosthesis

MATERIAL AND METHODS

One hundred and ten patients admitted to Medical De partment B during one year from February 1968 were examined, 65 cases with unoperated aortic valvular disease and 45 cases following insertion of a Starr Edwards aortic ball valve prosthesis. In 58 of the unoperated cases the clinical diagnosis was confirmed by left heart cathe terization with measurement of the systolic gradient across the aortic valves the degree of aortic regurgitation was determined by an independent observer performing cine angiography from the aortic root.

In the 65 unoperated patients aortic stenosis without insufficiency was found in 13 cases, and in 24 cases there was aortic insufficiency with a peak-systolic gradient less than 20 mm mercury across the aortic valves. Combined aortic stenosis and insufficiency was found in the re maining 8 cases the relative importance of stenosis and insufficiency not being evaluated. The preoperative diag nosis n the 45 operated patients was aortic stenosis in 13 cases, aortic insufficiency in ten, and combined aortic stenosis and insufficiency in 22 cases Minor mitral valvular les on was observed in a few cases Cases with mitral valvular lesion or other heart disease of significant importance were however not included in this study

Hemoglobin concentration blood cell counts serum iron concentrat on and total iron binding capacity serum haptoglobin concentration and the total serum lactic dehydrogenase activity were determined by routine laboratory methods The haptoglobin concentration deter mined as the hemoglobin binding capacity of the serum normally ranges from 30 to 180 mg 100 ml. We used a value of 15 mg/100 ml or less as an indication of s g nificant hemolysis In normals the total serum lactic

Table I *The distribution of the serum haptoglobin values in 65 cases with aortic valvular disease and 45 patients with a Starr Edwards aortic valve prosthesis*

	No of cases	Serum haptoglobin concentration (mg 100 ml)			
		< 15	15–50	50–100	> 100
Unoperated patients					
Aortic stenosis	13	1	1	4	7
Aortic insufficiency	24	4	3	7	10
Aortic stenosis and insufficiency	28	6	6	9	7
Operated patients					
With a Starr Edwards aortic valve prosthesis	45	37	3	3	2

dehydrogenase activity is less than 200 units liter Reticulocyte counts plasma hemoglobin and serum bilirubin concentrations were also determined. However as these data did not give additional information about the hemolysis the figures are not recorded here

RESULTS

The haptoglobin values are shown in Table I Low values indicating hemolysis were found in by 20% of the unoperated cases whereas frequency was about 80% in the operated cases The frequency of hemolysis in aortic insufficiency was about twice that seen in aortic stenosis In patients with combined aortic stenosis and insufficiency hemolysis was as frequent as in patients with pure aortic insufficiency Hemolysis was most often seen in patients with marked aortic insufficiency whereas it was rarely observed in cases with a slight aortic regurgitation However the correlation between the degree of aortic insufficiency and the occurrence of hemolysis was not good as several patients with marked aortic regurgitation showed no hemolysis No relationship between hemolysis and the degree of aortic stenosis could be demonstrated

In Table II the frequency of hemolysis in our patients is compared with data given by other authors Of the series published hitherto seven of the largest are included in the table In three series the life span of ^{51}Cr labeled red blood cells is determined and in four series the serum haptoglobin values are used as an index of hemolysis

Nearly half of the 45 operated patients were examined 2 to 4 weeks after the insertion of the aortic ball valve prosthesis In 15 of these patients the examination was repeated 2 to 8 months later and no significant change of the haptoglobin concentration was seen Therefore we suggest that an observation period of 3 to 4 weeks following the operation usually is sufficient to evaluate the occurrence of hemolysis Ball valve leakage due to suture insufficiency was seen in three cases their haptoglobin values being 5 10 and 15 mg/100 ml respectively Thus in these patients hemolysis did not seem to be more severe than in the rest of the operated patients

Fifteen patients were examined both pre and postoperatively Normal haptoglobin values were found in 13 and low values in two cases before the operation Reexamination 3 to 4 weeks after the operation showed a normal haptoglobin value

Table II *The frequency of hemolysis in the present series compared with figures given by other authors*

Author	Method	Unoperated		Operated	
		No of patients	Hemolysis	No of patients	Hemolysis
Brodeur et al. (2)	$^{51}Cr^{a}$	21	7 (33)	1[illegible]	6 (50*)
Westring (37)	$^{51}Cr^{a}$	1[illegible]	3 (5)		
Yacoub & Keeling (33)	$^{51}Cr^{a}$			15	8 (53)
Bell et al (1)	Hp^{b} 0	11	2 (18)	[illegible]7	0 (74*)
Cullhed (4)	Hp^{b} 13	6	2 (8*)	15	14 (93)
Dupont & Wennevold (6)	Hp^{b} 35	7	5 (18)		
Eysters et al (7)	Hp^{b} 0	1[illegible]	2 (18*)	12	4 (33*)
Present series	Hp^{b} 15	59	11 (18)	41	33 (8)

[a] Half life of chromium-labelled red blood cells Value indicating hemolysis arbitrarily set to 0 days or less.
[b] Serum haptoglobin concentration. Values used to indicate hemolysis by the different authors recorded in the table

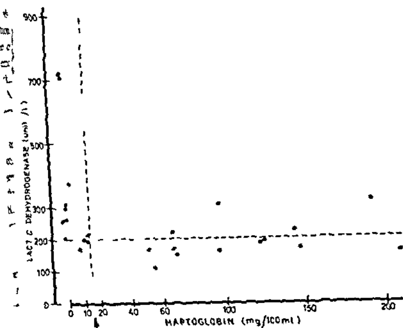


Fig 1 The relationship between the haptoglobin concentration and serum lactic dehydrogenase activity in 50 non-selected, consecutive cases.

in only one of these cases in the others there was a marked decrease of the serum haptoglobin indicating development of postoperative hemolysis

The total lactic dehydrogenase activity of serum was determined in 50 cases Fig 1 shows good agreement between these values and the serum haptoglobin concentrations The lactic dehydrogenase activity was increased to more than 200 units/l in all but one of 24 cases with hemolysis as determined by a serum haptoglobin concentration less than 15 mg/100 ml On the other hand in 26 patients with haptoglobin values exceeding 15 mg/100 ml the lactic dehydrogenase activity was less than 200 units/l in 20 cases In three cases the increase in lactic dehydrogenase activity was slight and in two of three cases with marked elevation of the lactic dehydrogenase the increase might be explained by a possible myocardial infarction in one and a serious heart failure in the other

Anemia was unusual even in cases with significant hemolysis as determined by serum haptoglobin and lactic dehydrogenase values Hemoglobin values between 10 3 and 13 0 g/100 ml were seen in 11 of the 65 unoperated cases only in one of these could the anemia be explained by hemolysis Irrespective of hemolysis iron deficiency was seen in several cases mainly in younger women In the operated patients anemia was more frequent However because of the short observation period following operation in this group the relationship between anemia and hemolysis was *not* evaluated No case of serious hemolytic anemia was observed in the present series

DISCUSSION

There is a high proportion of combined aortic stenosis and insufficiency in the present series of unoperated patients pure aortic stenosis being diagnosed in relatively few cases The reason for this probably is that heart catheterization was performed in the majority of cases thus giving a more precise diagnosis than the clinical findings alone

Our data about the frequency of hemolysis in unoperated and operated patients with aortic valvular lesions are in agreement with those of most other authors This may be surprising as different techniques and different normal values for serum haptoglobin have been used by some of the others However as the serum haptoglobin usually falls to values close to zero even in slight hemolysis borderline values are recorded in relatively few cases

In agreement with Brodeur et al. (2) we found that hemolysis occurred twice as frequently in aortic insufficiency as in aortic stenosis. However these authors found a significantly higher frequency of hemolysis in their unoperated patients, whereas their figures in the operated cases were considerably lower. Our rather short postoperative observation period did not seem to influence the results. Therefore we have no other explanation of the different frequency found in the two series than the different techniques used. Several authors have observed increased hemolysis in cases with leakage around the prosthesis (1, 2, 18, 22, 33) even in patients with heterograft aortic valves leakage invariably results in hemolysis (21). In our material ball valve insufficiency occurred in only three patients; in these the hemolysis was not more marked than in the rest of the operated cases.

The serum lactic dehydrogenase activity usually is increased in hemolytic states; this has also been observed in the hemolytic syndrome following aortic valve replacement (3, 19). Recently it has been pointed out (31) that the serum lactic dehydrogenase value seems to be a reliable quantitative index of hemolysis. We found elevated values in nearly all patients with intravascular hemolysis. But as expected there were increased values also in a few cases without hemolysis. The enzyme in these cases most probably originated from the myocardium, kidneys and liver. However our preliminary data (11) show a good correlation between the total serum lactic dehydrogenase value and the life span of red blood cells labeled with radioactive chromium. For clinical use we suggest that the haptoglobin value should be used to determine whether hemolysis is present or not, and that the serum lactic dehydrogenase activity is a simple and reliable parameter for evaluation of the degree of hemolytic activity. However the haptoglobin value shortly after blood transfusions and during serious infections such as bacterial endocarditis may be misleading. Furthermore care should be taken in interpreting the lactic dehydrogenase value, as myocardial, kidney and liver diseases have to be excluded before it can be used to evaluate the degree of hemolysis.

Other methods for determination of hemolysis, such as measurement of the carboxyhemoglobin in blood (9), serum bilirubin and plasma hemoglobin turnover, have failed in our hands. Others too (20) have reported failure of the carboxyhemoglobin method in such patients.

Sears and Crosby (24) observed a consistent relationship between physical activity and the rate of hemolysis in two patients. Recently this has been confirmed by others (8, 31). We have studied the degree of hemolysis before and after a graded exercise test in several patients (16). Irrespective of the hemolytic activity in the resting state a slight increase in hemolysis was observed during work in patients with a ball valve prosthesis. Further study is necessary to correlate the increase of hemolysis with hemodynamic parameters.

The mechanism of hemolysis in these patients is still uncertain. In most cases a mechanical trauma undoubtedly is the cause, but in some cases immunohemolytic anemia has been observed (13, 15). As to the mechanical trauma, there is no agreement about the relative importance of direct trauma between the red cells and the valves and the injury of the red cells caused by the turbulent blood flow (14, 19, 22). The markedly different frequency of hemolysis in patients with aortic stenosis and aortic insufficiency could possibly be explained by the high pulse amplitude in the latter condition causing increased mechanical stress on the red cells not only in the heart and aorta but also in smaller arteries.

Our preliminary data (11) suggest a shortening of the red cell life span to between 50 and 75 % of the normal, a finding in agreement with others (1, 2). Thus the shortening of the red cell life span is so moderate that the bone marrow will compensate for it if iron or folic acid deficiency does not develop. The present material of 110 consecutive non-selected patients did not include any case with anemia of clinical importance. Therefore we conclude that, despite a high frequency of hemolysis, severe anemia is seldom seen in patients with aortic valvular disease and in cases with Starr-Edwards aortic ball valve prosthesis.

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HYPOGLUCOSURIA AND L FORMS OF ESCHERICHIA COLI IN THE URINE

A Case Report

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Abstract. During screening for asymptomatic bacteriuria with the hypoglucosuria test, the urine from a seven-year old girl showed low glucose values, but negative conventional cultures Heavy growth of L forms of *Escherichia coli* unaccompanied by growth of the classical bacterial form, was obtained on two occasions. The girl had no symptoms, no past history of urinary tract infection, and had never been treated with antibiotics Shortly afterwards she presented symptoms suggestive of urinary tract infection, accompanied on this occasion by growth of classical *E coli*. The finding of subnormal fasting urinary glucose levels in combination with negative conventional cultures may indicate a latent infection due to L forms.

L forms represent bacteria which lack a rigid cell wall Because a rigid cell wall is lacking the L forms show pleomorphism and require a certain amount of osmotic protection in order to survive and multiply On agar media L form colonies are characterized by a center portion becoming embedded in the agar The center portion is surrounded by a peripheral growth on the agar surface which gives the familiar "fried egg" appearance After primary induction from ordinary bacteria the L forms retain their ability to proliferate but they are usually unstable since they tend to revert to the bacterial form The L forms may lose their tendency to revert after repeated subcultures (stabilized L forms)

Since the first report by Klieneberger in 1935 (20) L forms have been isolated in various human infectious diseases such as bacterial endocarditis (6 16 21) meningitis (17 22) and recurrent furunculosis (12) In 1965 Gutman et al (13) isolated L forms from the urine of 11 of 57 selected patients with chronic urinary tract infection and pyelonephritis Conner et al (7) found L forms in 26 of 115 patients with recurrent urinary tract infections However Gutman et al (14) have suggested that urinary tract infections, associated with L forms, are probably uncommon It has been proposed that the *milieu* in the renal medulla is osmotically favorable for the survival of L forms (4) Kalmanson and Guze (18) cultivated L forms from the renal biopsy material of seven patients. Five of these showed histological evidence of pyelonephritis The patient described below was one of a series of 511 school girls between 7 and 18 who were studied for bacteriuria (26 27) These studies and a previous investigation (25) showed that subnormal levels of urinary glucose i e less than 1 5-2 0 mg per 100 ml afford a reliable indication of significant bacteriuria A test paper was developed which was sensitive enough to give a color reaction for glucose levels down to 1 5-2 0 mg per 100 ml of urine The urine from eight of the 511 girls gave no color reaction with the test paper The glucose concentration of these eight samples was less than 1.5 mg per 100 ml Seven of the girls showed significant bacteriuria as defined by Kass (19) In contrast to these seven cases the urine from the patient reported on here yielded no growth of classical bacteria

METHODS

The patient followed the instructions used in the survey (26) for the collection of urine The patient had emptied her bladder on going to bed. Thereafter the patient had nothing to eat or drink until the sampling. The urine

v ted as a mid-stream specimen from the first, m r g urine

Urinary glucose

The glucose concentration of urine was determined semi quantitatively by the test paper method (17) and quantitatively by the hexokinase-glucose-6-phosphate method (18) Test papers (Unglox®) from AB Kabi, Stockholm 0 Sweden

Classical bacteria

Quantitative bacterial cultures were performed on blood agar plates by the calibrated loop technique

L forms

These were isolated on a solid medium heart infusion broth (Difco) 90 ml horse serum 20 ml yeast extract bakers yeast 25) 10 ml glucose 20 % 10 ml and sucrose 13 g Antibiotics were not included The pH was adjusted to 6 3 with hypochloric acid before adding 1 of agar to the medium. The plates were incubated at 37 C in anaerobic jars in an atmosphere of 90 nitrogen and 10 carbon dioxide Inoculated blood agar plates were incubated simultaneously in the same way to serve as controls. The plates were read under the microscope after 3 days of incubation and then every second day during a fortnight. Further observations were made under oil immersion, after agar blocks with growth of L forms had been prepared according to the wet-stained agar block technique (8) Subcultures were made by cutting out blocks of agar with growth of L forms The blocks were placed face down and passed over the surface of fresh plates For reversion of L forms to classical bacterial subcultures were made by the same technique blood agar plates

Serological typing of isolated E coli strains was done by Ida Orskov M D at the International Escherichia Center Copenhagen, Denmark.

CASE REPORT AND RESULTS

The patient was a 7 year-old girl Her past history was thoroughly investigated She had previously been in good health, apart from infections of the upper respiratory tract She had never been treated with antimicrobials, nor complained of symptoms suggesting urinary tract infection. Her family history was negative apart from the fact that a sister had Turners syndrome

The interval between the collection of the first two urine samples was 13 days. The urinary glucose concentration of the first sample was 0 6 mg per 100 ml and that of the second 0.5 mg per 100 ml. The pH was 6.3 and 5 6 respectively As there was no growth of classical bacteria in the first sample and the urinary glucose concentration was subnormal, repeated cultures from the same sample were made on blood agar plates, and cultures for L forms were prepared There was again no growth on the blood agar plates. On the L form medium, however developing colonies, visible under the microscope were found on the third day of incubation. The wet stained agar block technique showed pleomorphic growth Within the first week of observation heavy growth of colonies was noted. They were granular in texture with a dense center embedded in the agar (fried egg morphology) Classical bacteria were not found on the L form medium. The control blood agar plates showed no growth The colonies found could be propagated on L form medium by the agar block technique When colonies were passed, in the same way over blood agar plates, the organisms reverted to classical *E coli* after overnight incubation Thus the organisms primarily isolated on the L form medium represented L forms and, after reversion, they did not grow as L forms when subcultured on L form medium. Cultures for mycoplasma, including T-strains were negative

The results of cultivating the second sample collected from the patient were identical with those of the first sample Thus, in two separate samples collected 13 days apart, the primary cultures from the urine showed heavy growth of L forms but no growth of classical bacteria Also the L forms isolated from the second sample reverted to classical *E coli*

Two days after the collection of the second sample, the patient was requested to come to the Childrens Hospital for a clinical examination. She had at that time an obvious tenderness over the right kidney This tenderness had not been noted earlier by the patient. Otherwise the physical examination was normal Blood urea nitrogen was 13 mg per 100 ml WBC 3400 per cm³ ESR 3 mm per hour The first morning urine showed a glucose concentration of 0 4 mg per 100 ml and an osmolality of 11 mOsm per kg. Urinary sediments were normal. Intravenous pyelography and voiding urethrocystography showed normal conditions. In contrast to the samples collected earlier the conventional cultures of the urine yielded, at this time, growth of classical *E coli* with more than 100 000 organisms per ml. There was no growth of L forms. The *E coli* strain from this third sample and the two strains which had reverted from L forms had all the somatic antigen O 15 They were non motile They were also identical in fermentation of carbohydrates and showed the same pattern of sensitivity to antibiotics

In vitro the organisms were sensitive to sulfonamides and sulfamoxol was administered. Ten days later exanthema developed and the treatment was changed to nitrofurantoin Nitrofurantoin was given for 2 months. During the course of therapy and subsequently over another period of 18 months, repeated cultures yielded no growth of L forms or classical bacteria. The concentration of urinary glucose determined repeatedly during this period was always within the normal range and the test paper for glucose gave normal color reactions The patient had no symptoms suggestive of urinary tract infection and repeated physical examinations were normal.

DISCUSSION

L forms may be produced when bacteria lose the ability to synthesize a normal cell wall In vitro bacteria with a defective cell wall may be induced by antibiotics (11) lysozyme (32) microsomal fractions of leucocytes (3) and by antibodies

and complement (5) Also in vivo these and other host factors may account for the conversion of classical bacterial forms to L forms However in most instances where L forms have been isolated the subject had received antibiotics especially penicillins and often the parent bacterial form reappeared after the antimicrobial treatment was discontinued The case described had never been treated with antimicrobials before the L forms were isolated Among the conceivable factors accountable for the formation of the L forms lysozyme was searched for Repeated assays for lysozyme in the urine performed according to Parry et al (23) showed no measurable activity

Generally the defective cell wall makes the L forms dependent on a high environmental osmolality so as to escape lysis Braude et al (4) have stated that hypertonic urine may support survival of L forms and that the first morning urin is most suitable for isolation of L forms as the osmolality of morning urine is usually sufficiently high to prevent lysis That in our case isolation of L forms was successful may possibly be due to the specimens being collected from the first morning urine

The pathways for carbohydrate breakdown by L forms are less well defined Most information about the carbohydrate metabolism of L forms has been collected from studies of Proteus L forms According to Weibull and Beckman (33) the L forms have less oxidative activity than does the parent bacterial form On the other hand L forms seem to have *a greater* degree of glucolysis than the parent form In vitro glucose appears to satisfy th energy requirements of L forms (1) The subnormal levels of urinary glucose found in the case described indicate that L forms of *E coli* localized within the *urinary tract* make use of the small amounts of urinary glucose normally present

L forms of *E coli* may survive and revert in the renal medulla of experimental animals (2) The hyperosmolality of the renal medulla was presumed to protect the L forms Experimentally Dienes and Weinberger (9) Silberstein (29) Freundt (10) and Gutman et al (15) were not able to demonstrate pathogenicity of L forms per se The pathogenicity returned when the L forms reverted to classical bacterial forms (10) In the present case there were no symptoms of urinary tract infection when the L forms were isolated However when the L forms had reverted tenderness over the right kidney region was found Flank pain or tenderness is often known to be the presenting symptom of urinary tract infection in children 5–12 years of age (24 30) In the present case the flank tenderness in combination with bacteriuria suggests that the L forms primarily isolated gave rise to a dormant state of kidney infection which manifested when the L forms reverted to the parent bacterial form As Gutman et al (15) have pointed out however it is extremely difficult to relate L forms to human infections because the parent organisms are frequently isolated concomitantly with the L forms Therefore in order to be able to relate L forms to disease in the host they must be isolated unaccompanied by classical bacteria preferably in the absence of antibiotic therapy thus indicating that L forms alone are capable of inducing disease These qualifications were satisfied by the patient described

The technique for isolation of L forms is both laborious and time consuming The detection of subnormal levels of urinary glucose in the presence of negative conventional cultures appears to be a useful means of selecting cases for L form studies

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DIAMETER OF THE RENAL ARTERY AND KIDNEY FUNCTION IN PATIENTS WITH PARENCHYMATOUS RENAL DISEASE RENAL ARTERY STENOSIS AND RENAL CARCINOMA

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Abstract In 79 patients (50 with parenchymatous renal disease 19 with unilateral stenosis of the main stem of the renal artery and ten with renal carcinoma) the diameters of the renal arteries (D) were measured by renal arteriography and the effective renal blood flow (ERBF) the inulin clearance (C) and the para amino-h ppuric acid clearance (C_{PAH}) were measured by the spl t function technique

In the patients with single renal arteries and parenchymatous renal disease pos tive curvilinear covariations between ERBF C , C and D as well as D^2 were found. When the difference in function between the kidneys in the same ind vidual (per cent of mean value) was correlated with the corresponding difference in D significant, positi e linear covariations between ERBF C C_{PAH} and D as well as D were found in patients with single renal arteries and parenchymatous renal dis ase There was no correlation between the functional parameters and D in the kidneys with renal artery stenosis or renal carcinoma.

It seems that D is so closely related to the blood flow through the renal artery when the variation of D is secondary to modifications in the parenchyma, that it can be used as approximate estimate of renal function.

In experimental hydronephrosis in the dog the diameter of the renal artery decreases pari passu with the area of the frontal projection the weight and the effective plasma flow of the kidney (6) In man the area of the frontal projection of the kidney has been reported to show a "logarithmic" co ariation with the diameter of the renal artery (7) In patients with focal and diffuse kidney diseases the diameter of the renal arteries show a statistically significant covariation with the effective plasma flow of both kidneys (2)

With the intention of obtaining a more accurate description and a practical application of the relation between kidney function and the arterial diameter the function of the individual kidneys has been compared with the arterial diameter in this study The findings in disorders primarily affecting the renal parenchyma have been compared with the findings in renal artery stenosis which primarily affects the artery and in renal carcinoma, where the flow through the renal artery is shared between the cancer tissue and the remaining part of the kidney

MATERIAL

The study comprised 50 patients *with pa enchymatous renal disease* 19 patients with *stenosis of the main stem* of the renal artery and ten patients with *renal ca cinoma*

The relevant cl nical data of the patients with parenchymatous disorders are given in Table I. The diagnostic criteria are given elsewhere (3) As the renal artery is usually involved in general atheromatosis, the patients were grouped according to age and the presence of hypertension. Hypertension was defined as a blood pressure exceeding 1 0 100 mm Hg (cuff method) Eyeground changes exceed ng group II of Keith and Wagener were not present in this patient group

Ele en of the 19 patients with renal artery stenos s were men. The mean age in this group was 48 6 years (12 to 63 years) With the exception of one normal kidney all kidneys were supplied by s ngle renal arteries. Eighteen patients were hypertensi e eyeground changes corresponding to group III of Ke th and Wagener were seen in two patients, and bilateral oedema of the papilla was seen in one

Four of the ten patients with renal carcinoma were men. The mean ag in this group was 63 8 years (53 to 74 years) With the exception of one normal kidney all k dneys were supplied by single renal arteries Three patients were hypertensive pathological eyeground f nd ings were not present in this patient group On the bas s of the renal arteriogram the tumours wer classified as having 1) an abundant vascular supply including the

Table I *Relevant clinical data in patients with parenchymatous diseases*

The numbers of patients with unilateral renal disease are given in par nthes s

	Normotensives (<45 y)	Hypertensives (<45 y)	Normotensives (>45 y)	Hypertensives (>45 y)	All groups
Sex Age (no of patients/years)					
Male	8/25 5	5/32 2	2/56 5	3/50 3	18/34 9
Female	5/ 8 0	3/35 0	12/57 8	12/56 4	32/50 5
Patients with single artery bilaterally					
Diagnosis (no of pats)					
Pyelonephritis	4		8	7	19
Pyelonephritis and hydronephrosis	1			2	3
Hydronephrosis	1	2		1	4
Hypoplasia	2	1	1	1	5
Tuberculosis			1	1	2
Papillary necrosis		2			2
Hypertension		2		1	3
Patients with single artery unilaterally					
Pyelonephritis and hydronephrosis	1		2		3
Hydronephrosis	3			1	4
Tuberculosis				1	1
Hypertension		1	1		2
Patients with multiple arteries bilaterally					
Pyelonephritis and hydronephrosis			1		1
Tuberculosis	1				1
Total	13 (9)	8 (5)	14 (7)	15 (9)	50 (30)

es ke ... ... or 2) a moderate or sparse vascul r ... h ... erized by the presence of fewer ramificat ... ... arteries in the tumour than in the neighbou ... ... ue All tumours were extirpated. The tumou ... ... ted as having a moderate or sparse vascular sup ... either cystic (7 patients) or grossly necrotic

With the ex ... n ... f two patients (one with parenchymatous diseas ... nd ... ne with renal artery stenosis) the serum concentr ... ns of creatinine and urea were normal The patient ... d not present symptoms or signs of electrolyte imbal ... and were without signs of manifest heart failure

METHODS

The procedure and methods of analysis and calculation used in measurements of C ... C ... and ERBF of the *individual kidneys are described elsewhere (3)*

The abdominal aortography was performed by percutaneous catheterization via the femoral artery and injection of the roentgen contrast medium (30 ml of 70 ... Isopaque) by the Gidlund pump Six exposures were taken during the first 2½ sec after the injection, and *an additional exposure 10 sec after the injection of the* roentgen contrast medium.

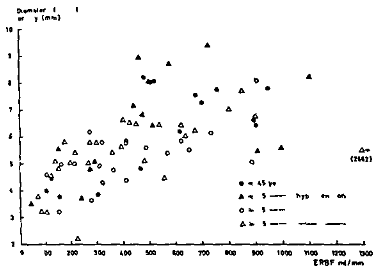
The following diameters were measured on the arteriograms 1) the diameter of the renal arteries (D ...) at the point of first division 2) the diameter of the abdominal aorta 1 cm central to the renal arteries 3) the diameter of the abdominal aorta 1 cm peripheral to the renal arteries and in patients with renal artery stenosis also 4) the minimal diameter of the stenosis Correction for the average magnification (1 17 times) of the true dimensions was not made All measurements were performed by the same investigator and by the use of the points of a pair of compasses mounted on a sliding guage A vernier scale on the sliding guage permitted readings of 0 1 mm On the basis of duplicate readings performed with an interval of several months the error of measurement ($\sqrt{d^2/n}$ d = difference between duplicate readings, n = number of duplicate readings) was found to be 0 43 mm for the diameter of the renal artery 0 64 mm for the diameter of the abdominal aorta and 0 40 mm for the difference between the diameters of the renal arteries of the same individual

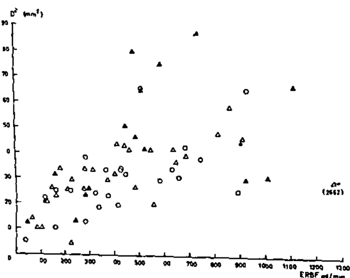
Conventional methods (5) were used in the statistical treatment of the results

RESULTS

Patients with parenchymatous renal disease and kidneys with a single renal artery

When C_{In} C_{IAH} and ERBF were plotted against D_{ra} or D_{ra} the distribution of the observations





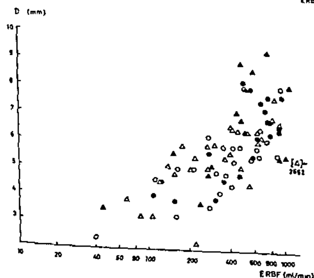


Fig. 1. Relation between diameter of renal artery (D and D) and effective renal blood flow (ERBF linear and logarithmic coordinates) in patients with parenchymatous disorders

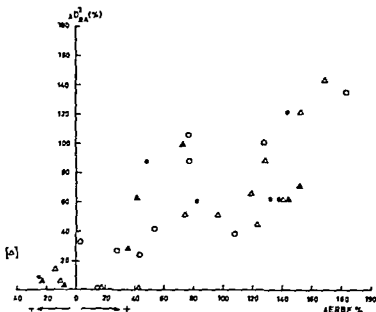


Fig 2 Relation between percentual difference of arterial diameter (D) and effective renal blood flow (ERBF) in the same individual in patients with parenchymatous disorders. Symbols as in Fig. 1

seemed to be curvilinear in the linear as well as in the semilogarithmic coordinate system (Fig 1 Table II) A common feature was an increased scatter of the observations when ERBF exceeded 450 ml min C_{PAH} exceeded 250 ml/min and C_I exceeded 35 ml min

Statistically significant correlations were found between C_I $C_{I\,AH}$ ERBF and D_{ra} as well as D^*_{ra} ($p<0.05$ Table II) No effect of hypertension or age was observed

When the difference in function between the kidneys of the same patient (per cent of mean value) was plotted against the corresponding difference between the arterial diameters (D_{ra} or D_{ra}

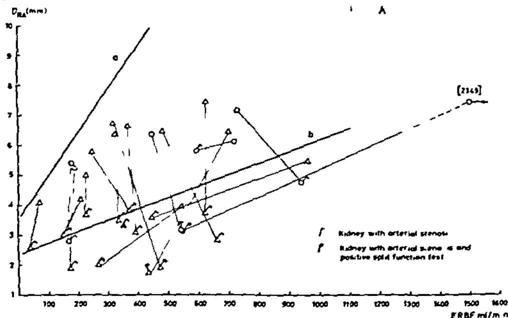
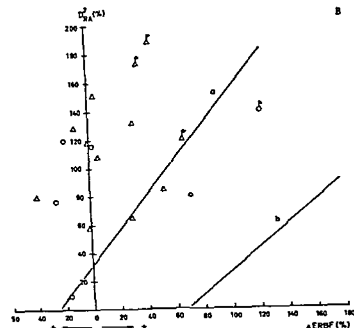
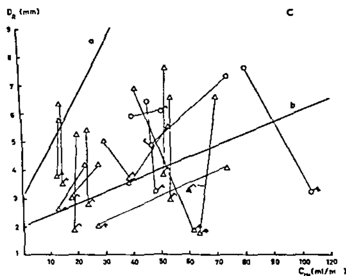


Fig 3 (*A*) Relation between diameter of renal artery (D_{RA}) and effective renal blood flow (ERBF) in patients with renal artery stenosis. (*B*) Relation between percentual difference of arterial diameter (D^2) and effective renal blood flow (ERBF) (*C*) Relation between diameter of renal artery (D) and inulin clearance (C) Symbol as in Fig. 1 *a* and *b* outline area of corresponding observations in patients with parenchymatous disorders.





F 3 B C

per cent of mean value) linear and highly signifi cant ($p < 0\ 01$) correlations were observed (Fig 3 Table II)

The function was reduced below accepted limits of normality (4) when D_{ra} was less than 4 mm (12 kidneys $C_I < 36$ ml/min $C_{PAH} < 190$ ml/min ERBF < 320 ml/min)

The kidneys with an arterial diameter of less than 3 5 mm were almost without glomerular filtration The function was normal or only slightly reduced when D_{ra} was greater than 6 5 mm (24 kidneys $C_I > 30$ ml min $C_{PAH} > 225$ ml/min ERBF > 400 ml/min) In the kidneys with 4 mm < D < 6 5 mm substantial reductions as well as great increases of the function were observed

Patients with parenchymatous renal disease and multiple renal arteries in one or both kidneys

Putting D_{ra} (or D_{ra}) as equal to the sum of the individual diameters (or the sum of the squares

Table II *Correlation between diameter of the renal artery and kidney function in patients with parenchymatous disorders*

Δ = difference between the kidneys of the sam individual (per cent of mean value)

y	x	Correlation coefficient	Regression equation
D	C_i	0 55	$y = 4\ 09 + 0\ 0389x$
D^2_{rn}	C_{In}	0 3?	$y = 25\ 4 + 0\ 26x$
D_{rn}	C_{PAH}	0 81	$y = 3\ 76 + 0\ 00795x$
D	C_{PAH}	0 72	$y = 18\ 27 + 0\ 0704x$
D	ERBF	0 53	$y = 4\ 27 + 0\ 00332x$
D^2	ERBF	0 32	$y = 26\ 47 + 0\ 0?0x$
ΔD	ΔC_{In}	0 81	$y = 8\ 13 + 0\ 287x$
ΔD^2_{rn}	ΔC_i	0 78	$y = 19\ 00 + 0\ 480x$
ΔD_{rn}	ΔC_{PAH}	0 71	$y = 9\ 79 + 0\ 265x$
ΔD^2	ΔC_{PAH}	0 75	$y = 18\ 64 + 0\ 482x$

of these diameters) in kidneys with multiple renal arteries gave no correlation ($p > 0\ 10$) between the difference in diameter (D_{rn} or D_{rn} per cent of mean value) and the difference in ERBF or C_i (per cent of mean value) between the kidneys of the same individual The deviation from the observations in patients with single arteries on both sides was greatest in the patients in whom one kidney was supplied by two renal arteries of about equal diameter

Patients with renal artery stenosis

When C_i C_{PAH} and ERBF were plotted against D_{rn} or D_{rn} the observations in kidneys without artery stenosis corresponded with the findings in patients with parenchymatous disorders (Fig 3) The observations in the kidneys with stenosed

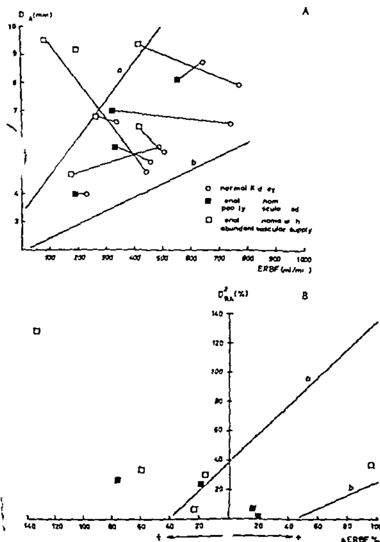


Fig 4 (*A*) Relation between diameter of renal artery (D) and effective renal blood flow (ERBF) in patients with renal carcinoma (*B*) Relation between percentual difference of arterial diameter (D) and effective renal blood flow (ERBF) in the same individual *a* and *b* outline area of corresponding observations in patients with parenchymatous disorders.

arteries differed from the latter showing no significant correlation between function and arterial diameter (D_{ra} or D_{ra}) At equal D_{ra} the blood flow in a stenosed artery was greater than in an artery without stenosis There seemed to be no difference between the observations in kidneys involved in a positive split function test (4 kidneys with stenosis) and the other kidneys with stenosis of the renal artery

Patients with renal carcinoma

When C_i C_{PAH} and ERBF were plotted against D_{ra} or D the observations in the normal kidneys were in correspondence with the findings in patients with parenchymatous disorders (Fig 4) The observations in four out of six kidneys with carcinoma with an abundant blood supply fell outside the range of observations in the group with parenchymatous disorders The ERBF in kidneys with carcinoma tended to be less than in a kidney without carcinoma at equal D_{ra}

Patients with parenchymatous disorders Difference between the aortic diameters 1 cm central and 1 cm peripheral to the origin of the renal arteries and ERBF

No significant correlation was found between the effective blood flow leaving the abdominal aorta through the renal arteries and the difference in diameter of the abdominal aorta central and peripheral to the origin of the renal arteries

DISCUSSION

The present investigation confirms earlier assumptions of a positive correlation between the diameter of the renal artery and the blood flow through the artery It would seem that this correlation is not absolute but depends on the modification in the renal artery being secondary to modifications in the parenchyma. The lack of correlation between function and diameter in the stenosis group is probably due to most of the stenoses not being particularly pronounced it is well known that slight stenoses have little haemodynamic effect On the other hand the lack of correlation between the diameter of the renal artery and ERBF C_{PAH} and C_i in the diseased kidney of cancer patients shows that the diameter of the renal artery is not a function of the amount of functioning renal tissue but of the blood flow through the artery in the kidneys in which the vascularization of the tumour was abundant ERBF was surely much less than the blood flow through the kidney

As the functional parameters were better correlated to D_{ra} than to D_{ra} the cross section of the artery does not appear to be the dimension that gives the best expression of changes in the blood flow through the kidney The findings in kidneys with multiple arteries point in the same direction

It seems reasonable to interpret the relation between the diameter and blood flow as an indication that the diameter of the renal artery depends on the amount of blood usually flowing through it The correlation found between the diameter and the blood flow will thus be affected by the investigation situation to the extent this causes a deviation from the usual blood flow of the kidneys Taken in conjunction with the relatively great measuring error in the diameter measurements this may explain the scatter of the observations especially at the highest ERBF values Unusual RBF in some patients during the selective function test may also explain why the correlation between the diameter of the renal artery and ERBF was curvilinear when the data for all kidneys were pooled (Fig 1) and linear when based upon the differences between the diameters and the ERBF of the kidneys in the same individual (Fig 2) If ERBF deviated from the usual flow in the same way in both kidneys in these patients the change of the percentual difference in ERBF would be relatively slight even on a considerable change in blood flow However unusual flow rates in some patients provided that the corresponding variation of D_{ra} was small might transform a linear correlation between ERBF and D_{ra} into a curvilinear one when the absolute flow rates are plotted against absolute values for D_{ra}

It seems that D_{ra} is so closely correlated to the inulin clearance and the blood flow through the kidneys in patients with parenchymatous disorders that D_{ra} in kidneys with single arteries can be used as an approximate estimation of the renal function in these patients A common problem in urology is the question whether a kidney should be resected or removed As a guiding rule kidneys with D_{ra} of less than 3 mm can be removed with a loss of only 10 ml of glomerular filtrate or less kidneys with D_{ra} of 6 mm or more can tolerate

an ordinary kidney resection of $^1/_3$ or $^1/_4$ of the kidney mass

The investigation cannot answer the question of why the diameter of the renal artery is reduced even in minor reductions of blood flow In view of the fact that the pathological modifications in several of the patients were unilateral it would seem reasonable to seek local causes In this connection it is of interest that Hilton (1) has demonstrated local modifications not of nervous origin in the volume of the femoral artery of the cat on modification of the peripheral blood flow in the extremity concerned

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SERUM B_{12} AND SERUM IRON AFTER GASTRIC SURGERY

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Abstract Immediately after Billroth II one third of patients have lost intrinsic factor most of them permanently As time goes on there is a steadily rising incidence 45% lacking intrinsic factor after 18 months Impaired absorption of labeled vitamin B_{12} is more frequent after surgery for stomach cancer than in patients with gastric or duodenal ulcer Loss of intrinsic factor is less pronounced in duodenal than in gastric ulcer

The pattern of serum iron levels gradually becomes similar to that of pernicious anemia after surgery All patients operated on should be kept under medical observation, so that vitamin B_{12} and iron can be supplemented at the most favorable time

The benefits of gastric surgery are many including elimination of life threatening complications such as perforation hemorrhage and stenosis In recent years however there has been a wider consideration of ill effects such as dumping bilious vomiting and hypoglycemia on the one hand and metabolic disorders such as vitamin B_1 hypovitaminosis including neurological and mental disturbances osteoporosis steatorrhea and hypoproteinemia

According to Deganello (2) Moynihan (9) and Hartmann (5) the complete picture of pernicious anemia after total gastric resection will appear after 2–3 years or even more As a first sign according to Pitney and Beard (10) the serum B_1 will diminish then follows macrocytosis and finally megaloblastic anemia

Earlier Lous and Schwartz (7) from Denmark in a study from 1959 of fifty-one patients with partial gastrectomy for duodenal ulcer found reduced absorption of vitamin B_1 in seven In 15 out of 45 patients operated on for gastric ulcer and 13 out of 17 patients operated on for cancer of the stomach the labeled vitamin B_{12} absorption test was impaired The average observation period was six years

In 1965 Deller (3) published a study comprising 365 patients The selection was made at random from hospital records and reexamination The male female ratio was 2 3 1 duodenal gastric ratio 1 4 1 and mean interval after surgery seven years Sixty-one had radioactive vitamin B_1 absorption tests and fifty seven bone marrow biopsies The marrow was megaloblastic in 2% and transitional megaloblastic in 5% A low serum vitamin B_1 level was found in 13% The absorption of labeled vitamin B_1 was impaired in more than one third The serum iron level was reduced in one third of subjects and anemia defined as a level less than 13 6 g% in men and 11 6 g% in women was detected in one-quarter Mainly then the anemia was due to iron deficiency although a deficiency of *vitamin* B_1 was also apparent in some cases

It might therefore be of some consequence if one could at an early stage foresee which patients are likely to develop vitamin B_1 hypovitaminosis after gastric surgery

MATERIAL

To fulfil this object, we have collected sixty-three patients (49 men and 14 women) operated on with Billroth II Twenty five patients had gastric ulcer (19 men and 6 women) including one woman with protein loosing gastritis Twenty-seven patients had duodenal ulcer (21 men and 6 women) Eleven patients had carcinoma of the stomach (9 men and 2 women)

METHODS

All patients underwent a series of blood examinations Schilling s test serum iron and vitamin B assay immediately after gastric surgery and every 6 months afterwards the last of them performed after two years.

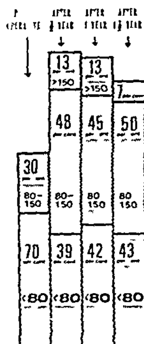


Fig 1 Percentage of serum iron values >150 μg 80 to 150 and less than 80 μg% post-operatively , 1 and 1 year after Billroth II

The labeled B absorption test was carried out according to Schilling s (13) urinary excretion method after oral administration of 0 5 μg of 57 CO-labeled vitamin B_{12}. One mg of cold vitamin B_{12} was injected 1 , h iter and the radioactivity of the 24 h urine specimen was measured with a well type scintillation counter EKCO 564 A

RESULTS

Twenty-one patients (18 men and 3 women) out of 63 (33° had a Schilling s test of 10% or lower immediately after gastric surgery Among those with impaired absorption of radioactive vitamin B_1 10 of 26 had been operated on for gastric ulcer 1 for protein loosing gastritis 5 of 26 for duodenal ulcer and 6 of 10 for cancer of the stomach In other words the risk of loss of intrinsic factor is far greater after partial gastrectomy for cancer or ulcer of the stomach than for duodenal ulcer These findings agree with the observation of Lous and Schwartz (7) albeit they maintain that the loss of intrinsic factor is a fairly established fact immediately after surgery in one third of patients. Three of twenty-one had already at this stage a low serum vitamin B_1 value less than 100 pg/ml Among the remainder only two had so low a value

After 6 months twenty six (21 men and 5 women) of 61 (43%) had a Schilling s test of 10% or lower

Thirteen patients with a normal Schilling s test immediately after gastric surgery presented a pathologic test whereas eight patients with a pathologic test (of 10 10 8 7 6 4 3 and 1) had a normal test after 6 months It would seem reasonable to suggest that the high number of pathologic tests immediately after gastric surgery could be explained by a pathologic test adready before the operation This suggestion must be refuted as we know that patients with duodenal ulcer have a higher and patients with gastric ulcer of the body of the stomach a lower secretion of intrinsic factor than normal but usually they do not lack intrinsic factor according to Doscherholmen (4) Moreover as many as eight patients changed from a pathologic to a normal test later on and it therefore seems likely to suppose that such patients due to the trauma inflicted by surgery lost their ability to produce intrinsic factor for a limited time

This should be taken with some reservation, however As a matter of fact five patients had a low serum B_{12} value immediately after surgery Two of them had a pathologic Schilling s test both postoperatively and during the whole period of observation The same holds true for another case Being a case of cancer of the stomach it is easier to understand that there may be a loss of intrinsic factor at the time of operation The remaining two patients had a normal Schilling s test immediately after gastric surgery and later on

There are ten cases of malignancy in this study Eight of ten had a pathologic Schilling s test two had not A pathologic Schilling s test therefore seems to occur more often in patients with stomach cancer than in the rest of the material studied here 18 of 52 with stomach or duodenal ulcer

Evidently there is a relatively high chance for patients with stomach cancer to develop a vitamin B_1 hypovitaminosis when operated on whereas this risk seems to be much less in patients with gastric or duodenal ulcer Ten of twenty-six operated on for gastric five of twenty-six for duodenal ulcer six of ten for cancer of the stomach had impaired absorption of labeled vitamin B_1 The loss of intrinsic factor at this stage

is remarkable for the group of cancer patients and somewhat more pronounced for gastric than for duodenal ulcer patients

After one year 31 of 51 (21 men and 10 women) 61% had a pathologic Schilling's test Ten of them were new cases who had presented a normal test at the previous two controls The proportion of impaired absorption at this stage was fourteen of twenty-one for gastric nine of twenty two for duodenal ulcer and eight of eight for stomach cancer There is a distinctly higher number with intrinsic factor loss in the gastric than in the duodenal ulcer group In the group of stomach cancer the whole group has impaired absorption of labeled vitamin B_{12}

After 18 months only forty patients have hitherto been reexamined eighteen of them 45% (11 men and 7 women) with a pathologic Schilling's test Four of them were cases with a previous history of a normal test. Three of them remained pathologic 6 months later whereas the fourth has not yet been reexamined At this stage (18 months) there is a still much higher loss of intrinsic factor in the gastric ulcer t n of nineteen than in the duodenal ulcer group three of fifteen Five of the six patients with partial gastrectomy for cancer had a pathologic Schilling's test.

Iron deficiency

In the present study a normal serum iron level (80 to 150 μg %) was found in 30% and subnormal level (less than 80 μg%) in 70% post operatively The incidence of subnormal serum iron level therefore is much higher than the incidence of pathologic Schilling's test and of subnormal B_{12} vitamin level in the blood (Fig 1)

After 6 months a normal serum iron level was found in 48% a subnormal level in 39% and a high level (more than 150 μg%) in 13% After one year the corresponding levels are 45 42 and 13% and after 18 months 50 43 and 7% After two years we have hitherto got 24 controls only too few to permit definite conclusions There seems however to be a tendency to fewer high levels than before whereas the number of normal and subnormal levels is about the same as after 6 months and 1 year

On the whole we may conclude that there is a definite tendency to higher normal and high serum iron levels and a lower number of subnormal values after 1/2 1 and 1 1/2 year and probably a tendency to lower high levels later on

It is well known that the serum iron level is definitely increased in untreated cases of pernicious anemia After treatment there is a sharp drop of iron level to normal or even very low levels during the first 24 to 48 hours (6 8 14)

It seems reasonable to suppose that the behavior of the serum iron after gastric surgery reflects this specific tendency to establish the picture usually found in pernicious anemia a tendency which if registered in patients after Billroth II is a warning to institute proper treatment This tendency in our study is evident in spite of a few cases in which treatment with vitamin B_{12} was started as a consequence of low B levels registered initially or later during this study

DISCUSSION

In conclusion we may state as a most important finding that immediately after Billroth II not less than one third of the patients have a Schilling's test of 10% or lower and in thirteen of sixty three the test remains low later on On the other hand in eight cases a pathologic Schilling's test immediately after gastric surgery becomes normal later on

After 1/2 1 and 1 1/2 year there is a higher incidence of pathologic Schilling's test from 33% immediately after gastric surgery to 43 after 1/2 year 61 after 1 year and 45% after 1 1/2 year In other words after 1 1/2 year about 45% of patients in our study lack intrinsic factor and therefore are inclined to develop pernicious anemia. This tendency seems to continue for two years but due to the limited number of observations (22 only) we refrain from further evaluation

It might be suggested that a certain number of patients operated upon may have a pathologic Schilling's test and low serum vitamin B_{12} levels before the operation Except for a few cases this seems highly improbable In some instances operational trauma may favor a transitional loss of intrinsic factor

Impaired absorption of labeled vitamin B_{12} is more frequent in patients with stomach cancer than in patients with gastric or duodenal ulcer After 1 and 1 1/2 year practically all cases of stomach cancer still alive have lost their intr factor As time goes on the

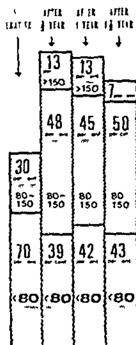


Fig 1 Percentage of serum iron values >150 μg 80 to 150 and less than 80 μg post-operatively ½, 1 and 1½ year after Billroth II

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RESULTS

Twenty one patients (18 men and 3 women) out of 63 (33%) had a Schilling's test of 10% or lower immediately after gastric surgery. Among those with impaired absorption of radioactive vitamin B_1 10 of 26 had been operated on for gastric ulcer 1 for protein loosing gastritis 5 of 26 for duodenal ulcer and 6 of 10 for cancer of the stomach. In other words the risk of loss of intrinsic factor is far greater after partial gastrectomy for cancer or ulcer of the stomach than for duodenal ulcer. These findings agree with the observation of Lous and Schwartz (7) albeit they maintain that the loss of intrinsic factor is a fairly established fact immediately after surgery in one third of patients. Three of twenty-one had already at this stage a low serum vitamin B_1 value less than 100 pg/ml. Among the remainder only two had so low a value

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PLASMA CELL NEPHROPATHY WITH RENAL TUBULAR ACIDOSIS

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Abstract A case of renal tubular acidosis in a 34 year-old man with electrolytic disturbances nephrocalcinosis and osteomalacia, and a long history of hypergammaglobulinemia with a positive Waaler Rose test is described. Surgical biopsy showed plasma cell infiltrates in the kidney It is suggested that renal tubular acidosis might sometimes be a manifestation of an autoimmune disease plasma cell nephropathy

The clinical picture of renal tubular acidosis was first described in the mid 1930s (3 9) since when the condition has also been seen in adults In most of the cases the gamma globulin concentration was also raised (1 16) Co-existing purpura hyperglobulinemica of the type described by Waldenstrom in 1944 (14) has also been reported (4 7) After tubular defects had been demonstrated in Sjögren s syndrome (8 11) and in myelomatosis (5 10) the capacity of the kidneys to acidify the urine in the presence of hyperglobulinemia was studied systematically by Curtis Morris Jr and Fudenberg (6) In several cases of hypergammaglobulinemia of varying etiology they found that the ability of the kidney to acidify the urine was reduced The mechanism of this disturbance was obscure

The histological changes in a case seen at our department might perhaps provide a clue to the relation between hypergammaglobulinemia and renal tubular acido is at least in polyclonal gammapathy This case is reported below

CASE REPORT

The patient was a man born in 1929

His father born in 1898 and his mother born in 1905 enjoyed good health, and at examination in 1968 the concentrations of the serum gamma globulins and electrolytes were normal.

During adolescence the patient appeared to be slightly mentally deficient. He felt well until 1955 when he developed diffuse abdominal pain for which he sought advice at the department. The only noteworthy findings were hypergammaglobulinemia (1 9 g/100 ml) and a positive Waaler Rose test. In 1964 he had an acute attack of left-sided abdominal pain. Urography then showed nephrocalcinosis. The hypergammaglobulinemia was unchanged. Serum creatinine 1.3 mg/100 ml. Serum bicarbonate 18 mEq/l. Serum potassium 3 1 mEq/l. Specific gravity of urine 1 007 in concentrated samples. Alkaline phosphatase 28 units. Repeated attacks of renal colic until 1967 In Feb 1967 vomiting, muscle weakness and abdominal pain supervened, and the patient deteriorated rapidly On admission to the Medical Department the patient was very ill with muscular adynamia and paralytic ileus. B P 125/80 Serum electrolytes (mEq/l) sod um 138 potassium 1 8 chloride 119 bicarbonate 10 calcium 4 0 Phosphorus 0 7 mg/100 ml pH of urine 7 0 pH of blood 7.30 No demonstrable aldosterone in urine Alkaline phosphatase 15 units. Serum creatinine 1 1 mg/100 ml, Hb 9 6/100 ml ECG showed grave electrolytic disturbances. After correction of these abnormalities the patient improved promptly and gained 15 kg within a few months. H s weight was then normal. Supplementary examinations were carried out during the period of clinical improvement.

Kidneys

Polyuria, about 3 1 a day Specif c gravity after restriction of fluid 1 009 pH of urine repeatedly about 7 0 No fall of pH after administration of ammonium chloride in a dose of 0 1 g kg body weight. As a rule the urine contained no protein or bacteria A stone was passed on one occasion. Analysis of stone calcium, phosphate and oxalate No glycosuria. Examination of amino acids revealed nothing remarkable

Aldosterone in urine 0–5.2–14 8 μg/day Creatinine clearance 70 ml/min. Phosphate reabsorption 75% Roentgen examination of the kidneys showed as before nephrocalcinosis.

Skeleton

Biopsy specimen of the iliac crest after admin tetracycline revealed severe active renal os

Fig 1 Dense inflammatory cellular infiltrates in the cortex Some tubules are atrophic and some contain lloid material A few glomeruli are atrophic H E, ×53

e ting fibro-osteoclasia Deposition of tetra xtremely scanty in relation to the widespread ses (Prof E. Uehlinger)

f the calcium metabolism with ⁸⁵Sr showed al l rance increased accretion and an t of exchangeable calcium. Roentgen exami e l n osteom la ia with certainty

r

rema with Hb of about 11 g/100 ml n rmal B e marrow of normal com u ation of the iron metabolism with ⁵⁹Fe d n ine remarkable total iron metabolism in the mg kg body weight da incorporation 100% in 9 d ys Vitamin B d fol acid in the blood

De e

Free HCl g1 a jun l in feces 3 g day Xylose and la tose te t normal Schilling test 17 excretion in 24 h l er t opsy n rmal tissue

Serological ar ysis

Gamma globulins ab ut 2 g 100 ml in several specimens (increased poly lonal G) Waaler Rose 1 8 FIIA reaction 1280 Immun flu rescen e examinati n positive for cell nuclei and smooth muscle and negative for thyroid, parietal cells and kidne

Renal bi psy

Surgical biopsy with remo al of two pieces from the lower pole of the right kidney was done b Dr Lind stedt, Urological Dept., La arettet Lund. The specimens were examined histologically b Prof F Linell

The specimen consisted f cortex nly The most salient findings were numer u interstitial cell infiltrates built up mainly of plasma cell with a moderate admixture of lymphocytes. Many of the infiltrates were confined to the superficial part of the cortex but some extended cord like into the deeper layers of the tissue Most of the infiltrates were situated around glomeruli. There were glomerular changes in the infiltrated areas. The specimen showed all stages of periglomular fibrosis, which in some areas encroached upon the glomeruli some of which had undergone hyalinisation Adjacent to the infiltrated areas were atrophic tubules, some of which contained colloid like masses. In some areas there were signs of urinary reflux with concentrated urine in partly widened ducts Around these areas was a moderate proliferation of fibroblasts and incipient formation of giant cells in the tubular epithelium. Interstitially there were a few calcium granules but otherwise no signs of nephrocalcinosis. Between the abnormal areas were large regions of well preserved cortical parenchyma with essentially normal glomeruli and well preserved tubules No obvious vacuolisation of the cells of the tubuli No angitis (Figs 1 and 2)

Course

During follow up (about two years) the patient's general condition and working capacity were good He occasionally had brief spells of hematuria in connection with the passage of stones. Apart from mild hyperchloremia the acid base balance was controlled by admini tration of alkalising salts (potassium sodium lactate) The blood pressure was normal (Table 1)

DISCUSSION

In our case hypergammaglobulinemia and a positive Waaler Rose test were demonstrated when the patient was 27 By the age of 34 he had nephrocalcinosis and 3 years later he had frank renal tubular acidosis No evidence of rheumatic disease of liver cirrhosis or of Sjogren s syndrome could be demonstrated There were no general manifestations suggesting SLE Neither were there any manifestations of purpura The most striking

Fig 2 Detail of Fig. 1 Dense infiltra e sisting of plasma cells. H E, ×340

Table I *Chemical findings and body weight 1955–1968*

Therapy started Feb 1967

	1955	1957	1964	1967 Feb	1967 April	1968 Dec
γ glob g/100 ml	1 9	..1	1 7	1 3	1 9	2.1
Hb g/100 ml	12 8	12.5	11 0	9 6	9 6	11 8
NPN mg/100 ml	30	27				
Creat. mg/100 ml			1 3	1 8	1 1	1 2
K mEq/l			3 1	1 8	4 2	4 1
Na mEq/l				138	144	145
Cl mEq/l				119	112	111
Bicarb mEq/l			18	10	20	19
Ca mEq/l			4 5	4 0	4 6	4 5
Alk phosph. units			25	8	15	7
Body weight kg	72	69	66	52	69	69

finding was the plasma cell infiltrates in the kidney

Renal tubular acidosis appears as a tubular syndrome of varying origin It may often be due to a genetic defect Occasionally it may perhaps be ascrib d to a toxic injury such as when it occurs after administration of tetracycline that has been stored too long (15) It is more difficult to explain the cases that develop in association with gammapathy The possibility of the increased amount of gamma globulin injuring the tubules either directly or via angitis has been considered In our case it would appear that the condition was due to tissue injury of the type seen in plasma cell hepatitis chronic thyroiditis and Sjogren s syndrome The actual clinical picture of renal tubular acidosis could then be conceived as a manifestation of renal injury of varying etiology It appears natural to compare it to myxedema for example as an expression of different kinds of injury of the thyroid or of hepatic insufficiency with its varying etiology The frequency of this type of mechanism in renal tubular acidosis is not known But it may be suspected in all cases of gammapathy Plasma cell infiltrates in the kidney can only be demonstrated after surgical biopsy fine needle biopsy not giving sufficient information on the radial deposition of plasma cells No cases with such infiltrates have been published possibly because of insufficient material available for examination Infiltrates of plasma cells and lymphocytes have been demonstrated at autopsy of one case of Sjogren s syndrome (2) But the report of that case said nothing about the acidifying capacity of the kidney Bucher and Reid (2) also reported the autopsy findings in ten other cases in some of which the histological picture resembled that in our case but no mention was made of a correlation between tubular function and the histological finding In a series of nine cases with Sjogren s syndrome kidney biopsies were studied in eight and one post mortem (13) Six of these patients had changes indicating interstitial nephritis and in one of them it was stated that latent renal tubular acidosis was present Another series (12) with 12 patients of the same syndrome contained one patient with glomerulonephritis and four renal tubular acidosis Five cases were biopsied and interstitial chronic inflammation was found in several preparations with numerous mature lymphocytes

ACKNOWLEDGEMENT

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IDIOPATHIC PULMONARY HYPERTENSION IN TWO SIBLINGS

Clinical Microangiographic and Histologic Observations

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Abstract The clinical course and patho-physiologic features of idiopathic pulmonary hypertension in two female siblings aged 26 and 22 years are reported. The pulmonary vascular lesions were studied in serial histologic sections and a morphometric method was used for the determination of medial hypertrophy in the muscular pulmonary arteries. Microangiographic examination of the pulmonary arterial system was also done in one case The pulmonary vascular lesions were similar in both cases, with prominent medial hypertrophy in muscular pulmonary arteries, as well as necrotizing arteritis, cellular intimal proliferation with plexiform lesions, and segmental fibrous obliteration. The alveolar capillaries were normal or in places dilated, and there was microangiographic and histologic evidence of collateral arterial supply of the alveolar capillary bed peripheral to obliterated segments of the pulmonary artery

This report includes the first microangiographic description of the plexiform lesions, which could be identified as tortuous vascular channels some of which did not allow the further transmission of contrast medium.

Idiopathic pulmonary hypertension (IPH) is a comparatively rare condition particularly affecting females and usually *fatal within a few years after the initial* symptoms The familial occurrence of this disorder has been reported in several communications (5 14 26) Histologically the pulmonary vascular lesions of IPH include medial hypertrophy cellular intimal proliferation intimal fibrosis, plexiform and dilatation lesions and necrotizing arteritis (31) These features are generally considered to be identical with those of pulmonary hypertension secondary to congenital heart disease Some authors however have claimed that developmental defects in the muscular pulmonary arteries form the underlying cause of the progressive vascular changes in IPH (6 8)

Angiographic studies of the pulmonary arterial system in IPH have revealed a pruned appearance of the peripheral pulmonary arteries (6) The fine intralobular arterial pattern however cannot be readily demonstrated by conventional angiographic techniques

The purpose of the present paper is to describe the clinical course and patho-physiologic features of IPH in two siblings and to report the results of combined microangiographic and histologic studies of the intralobular arterial pattern in one of these cases

CASE REPORTS

Case 1

A Latvian woman, first admitted to the hospital at the age of 25 Two years earlier a systolic murmur had been heard over the pulmonary area at a routine examination. Because she felt asymptomatic she deferred further examination at that time She now complained of exertional dyspnea and easy fatiguability gradually worsening over the last eight-ten months. The patient also complained of syncopal attacks on exertion and of dizziness on standing up She gave a history of moderate edema of her feet during the last few weeks. There was no history of recurrent attacks of cough or fever and there was no past history of joint pains

An elder sister (case 2) had died two years earlier of primary pulmonary hypertension. No other case of either pulmonary hypertension or other remarkable cardiovascular or congenital diseases were known in her family

On examination at rest she showed no dyspnea or peripheral cyanosis The jugular veins were not distended, and there was no hepatic enlargement. The second heart sound at the pulmonary area was split, with accentuation of the pulmonary component. There was a loud pulmonary ejection murmur but no diastolic murmur A marked right ventricular heave was present. There was no radiological evidence for cardiomegaly but there was a huge dilatation of the pulmonary conus with markedly oligaemic peripheral lung fields Electrocardiogram had

Table 1 *Pressures (mm Hg) cardiac output (l/min) and stroke volume (ml) during rest and exercise supine position*

		Right ventricle		Pulmonary artery			PCV	Aorta			Cardiac	Stroke
		S	De	S	D	M	M	S	D	M	output[a]	volume
Case 1												
Rest	11 mo before death	89	6 5	87	45	60	9	113	71	90	4 8	54
Rest	6 mo before death	98	10	100	53	73		114	88	94	3 8	50
Case 2												
Rest	10 mo before death	95	10	97	48	66	6	102	72	83	4 5	47
00 kpm min	10 mo before death	151	26	151	70	102	9	119	70	88	5 3	41

[a] Calculated according to the Fick principle

PCV pulmonary arterial wedge pressure S = systolic D = diastolic De = end-diastolic M = mean. Reference point for all [illegible]ures at mid-dorsoventral chest level

[illegible] right ventricular hypertrophy and tall *P* waves [illegible]odynamic data obtained with right heart cathe[illegible]e listed in Table I There were markedly [illegible]ures in the right ventricle and pulmonary [illegible]n pulmonary capillary venous pressure [illegible] here was a significant elevation of the [illegible] esistance There were no signs of [illegible]cation between the right and left

[illegible]heterization one month after ad[illegible] [illegible] it was possible to reduce the [illegible]e considerably by the in[illegible] (CIBA 51531 Ba a pyra[illegible]vative) At [illegible]in unchanged or moder[illegible] [illegible]diac output 3 2–3 7 *l/min) and no* [illegible]e in the [illegible] arterial pressure the [illegible] fell from 58–61 mm Hg [illegible]e of 22–35 mm Hg within [illegible] injection of 50 mg of the dilato[illegible] however the maximal pressure [illegible] *to 60 mm Hg,* and the re[illegible]n the first occasion (return to cont[illegible] [illegible]thin 3 min as compared with 10–15 min 4 [illegible]nths [illegible])

During the f[illegible]llow[illegible] months there was a progressive worsening of the patient [illegible]dyspnea and general fatigue *Syncopal attacks foll*[illegible] exert[illegible]n became increasingly frequent. Repeated resp[illegible]ratory infections occurred in spite of continuous treatment with antibiotics *The hemodynamic* data obtained 6 months before her death are shown in Table I, together with those obtained 5 *months* earlier Nine months after the first examination a greatly enlarged heart was detected radiologically (1240 ml – 750 ml/cm surface) Two months later the heart volume attained 1350 ml – 830 ml m body surface Signs of hepatic enlargement, ascites and *prominent peripheral* edema appeared finally She died from right ventricular failure at the age of 26, eleven months *after she was* first seen in the hospital

The major autopsy findings were limited to the thoracic organs. There was prominent dilatation and hypertrophy of the right side of the heart affecting both the ventricle and the atrium. The weight of the heart was 400 g (body weight 52 kg) The right ventricular wall was 1? mm thick and the right atrial wall ranged up to 4 mm. There was no cardiovascular malformation. Some atheromatous plaques were observed in the pulmonary trunk. The main branches of the pulmonary artery were left intact at autopsy for subsequent injection. The angiographic and histologic studies (see below) did not give evidence of pulmonary thromboembolic disease

In the upper lobe of the right lung near the hilus there was a rounded cystic lesion measuring about 3×2×2 cm, with a fibrous wall and containing calcified amorphous material The nature of this lesion could not *be clarified, but the histologic picture was suggestive of* inactive tuberculosis There were no granulomatous changes or other evidence of active inflammation in this area. The vascular lesions in the lungs will be described separately below In the pulmonary parenchyma proper there were no significant gross or histologic changes.

The remaining organs were congested, but otherwise essentially *normal*

Case 2

A sister of case 1 born two years earlier The symptoms and physical findings when she was admitted to the hospital ten months before her death were closely simila[illegible] to those of her sister as was also the progress of her disease. *There was a loud systolic murmur* over the third and fourth interspaces at the left sternal edge a heaving left parasternal impulse and electrocardiographic signs of right ventricular hypertrophy In angiograms the pulmonary arteries were very wide centrally and strikingly narrow towards the periphery The passage of the radioopaque injectate through the pulmonary circulation was slow *The hemodynamic findings* obtained with right heart catheterization one month after admission to the *hospital are shown* in Tables I and II. The arterial oxygen saturation showed a decrease dur[illegible]g light muscular exercise There were no signs of a right-to-left shunt.

Recurrent respiratory infections were not *as frequent*

Table II *Data obtained with heart catheterization during rest and exercise in case 2 (supine position) 10 months ante mortem*

	O_2 uptake (ml min STPD)	O_2-capacity (vol %)	O_2 sat ( )		a-$\bar{v}$ O_2 diff (vol)
			Pulm. art	Aorta	
Rest	?49	18 0	72	99	5 5
00 kpm/min	648	18 1	62	91	12 3

in this patient as in her sister but hepatomegaly ascites and peripheral edema finally became even more prominent. There were syncopal attacks but not as frequently as in case 1 The patient died in right ventricular failure at the age of 2?

The main autopsy findings were confined to the heart and the pulmonary arterial system. As in the former case there was prominent hypertrophy and dilatation of the right ventricle and atrium, but no evidence of cardiovascular malformation The weight of the heart was not recorded The pulmonary trunk and the main branches of the pulmonary artery were dilated and the site of wide-spread atheromatous lesions. In the right upper lobar branch of the pulmonary artery there was a yellowish-grey thrombus, firmly adherent to the vascular wall, probably an organized embolus The lesions of the peripheral pulmonary arteries will be described separately below The pulmonary parenchyma proper was dry brownish, without infarcts or inflammatory changes

There was a small peptic ulcer in the stomach, at the lesser curvature close to the pylorus The remaining organs were congested but otherwise unremarkable

MICROANGIOGRAPHIC AND HISTOLOGIC STUDIES

Methods

The lungs of case 1 were injected for 30 min with 75% aqueous suspension of fine barium sulfate (Micropaque® Damancy & Co) The injection pressure was recorded and kept at 80 mm Hg. The filling of the lungs was checked in survey radiograms. After fixation in neutral formalin, frontal slices (2–3 mm thick) of the lungs were radiographed and representative specimens from all lobes were selected for microangiography The selected specimens were embedded in a mixture of histowax and beeswax and cut in slices 1000 μ thick, which were stereomicroangiographed by a technique described elsewhere (24) Abnormal features found in the microangiograms were correlated to the histologic appearance of the same vessels in serial sections Areas chosen for serial sectioning were cut out of the larger blocks and re-embedded in paraffin. The histologic sections were stained with hematoxylin and eosin or with Weigert s elastic tissue stain combined with van Gieson s stain.

From case 2 only two paraffin-embedded blocks of lung tissue were available These were studied in serial histologic sections stained as in case 1

The medial thickness of the muscular pulmonary arteries was measured according to the principles suggested by Wagenvoort (30 31) This morphometric method yields a mean ratio between the medial thickness and the external diameter of the arteries

RESULTS

Angiographic and microangiographic findings

In survey angiograms of the whole lungs of case 1 the peripheral pulmonary arteries were tortuous and there was poor capillary filling of the pulmonary parenchyma The microangiographic pattern was essentially the same in all lung lobes with prominent irregular narrowing or total obstruction of numerous intralobular arteries (Fig 1) A few plexiform lesions were visualized in the microangiograms as tortuous vascular channels (Fig 2) some of which did not allow further transmission of the contrast medium



Fig 1 Microangiogram (case 1) showing prominent, irregular narrowing of the intralobular arteries and poor capillary filling of the pulmonary parenchyma ×9

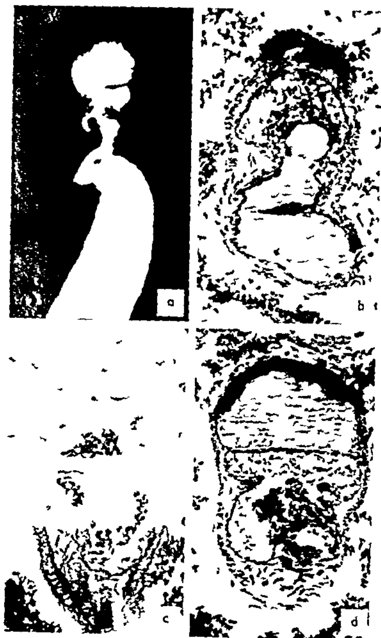


Fig 2 Plexiform lesion demonstrated in microangiogram and serial sections. (*a*) Microangiogram × 64 The plexiform lesion stands out as a tortuous appendix to a muscular pulmonary artery (*b–d*) Selected serial histologic sections from upper area in (*a*) showing various levels of the plexiform lesion There is some transmission of contrast through the plexiform lesion to a dilated small muscular pulmonary artery (*c* and *d* upper) × 162. (*b* and *d*), Hematoxylin-eosin (*c*) Elastin–van Gieson.

Patchy filling of the alveolar capillary bed was demonstrated in the *microangiograms* even in som areas cut off from their usual arterial supply Overdistens on of capillaries and precapillaries in the neighborhood of such areas possibly related to collateral circulation (see below) caused an irregular bushy appearance of the microangiographic pattern (Fig 3)

The contrast medium had reached the bronchial arterial system in many places probably via arterial bronchopulmonary anastomoses Three such anastomoses were found in the microangiograms and later verified by serial sectioning Two of the anastomoses were pleural and of the end to-end type One of these (lumen diameter 320 μ) was located at the mediastinal aspect of the left upper lobe and the other (diameter 200 μ) at the lower interlobar fissure of the right lung The third anastomosis (diameter 100 μ) was intra pulmonary of the end to-side type and situated in the right middle lobe

Histologic and morphometric findings

The histologic appearance of the pulmonary vascular lesions were essentially similar in case 1 and case 2 There was considerable medial hyper

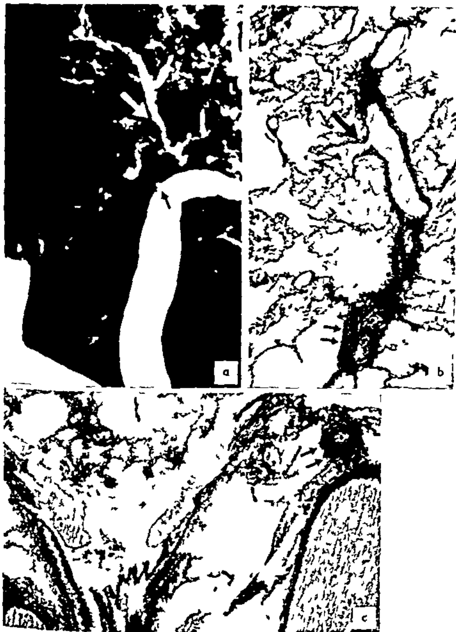


Fig 3 Intralobular collateral arterial supply of the alveolar capillary bed peripheral to an obliterated segment of the pulmonary artery The origin of the obliterated branch is indicated by small black arrow in *(a)* and the same artery by small black twin arrows in *(b* and *c)* Peripheral to the obliterated segment, the artery is patent and filled with contrast *(b* right upper) By means of arteriolar branches (e g corresponding arrows in *a* and *b*) the artery communicates with the precapillaries and the alveolar capillary network *(b* center) The latter is also supplied by intralobular capillary precapillary communications, which give the "bushy" appearance of the microangiogram in the right upper part of *(a)* The overdistended bronchiolar precapillaries in *(a)* left center and *(c)* left center probably also represent intralobular collateral communications, though unrelated to the arterial supply of the capillary bed visible in this particular field. *(a)* Microangiogram, × 27 *(b* and *c)* Selected serial histologic sections from central area in *(a)* Elastin–van Gieson × 69

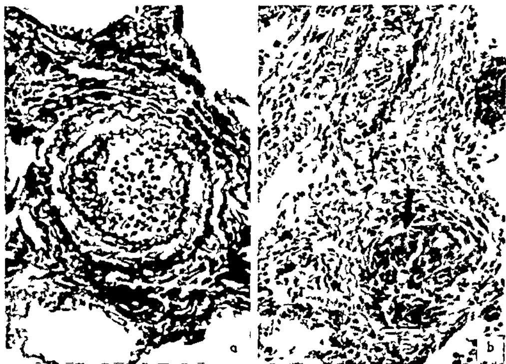


Fig 4 Pulmonary vascular lesions case 1 (*a*) Medial hypertrophy in muscular pulmonary artery with complete fibrous obliteration Note the presence of lymphocytes in the fibrous tissue obliterating the lumen. Elastin–van Gieson ×250 (*b*) Muscular pulmonary artery running from top and dividing in the center of the field Intimal fibrosis and medial necrosis in the parent branch The left lower branch is completely obliterated by loose fibrous tissue There is some perivascular lymphocytic infiltration and some deposition of anthracotic pigment. In the right lower corner there is a plexiform lesion with focal fibrinoid necrosis (arrow) Hematoxylin-eosin ×182

ophy in the majority of the muscular pulmonary arteries (Fig 4 *a*) The morphometric studies showed a medial thickness external diameter index of 10% (normal 5%) in case 1 The corresponding index in case 2 was 13%

Cellular intimal proliferation with multiple plexiform lesions was a prominent feature in both cases (Figs 2 4 *b* and 5 *b*) Focal fibrinoid necrosis was observed in the media of muscular pulmonary arteries (Fig 5 *a*) in the cellular intimal proliferations and in the plexiform lesions (Figs 4 *b* and 5 *b*)

There was prominent irregular intimal fibrosis largely concentric in type in muscular and transitional (elastic muscular) pulmonary arteries with complete obliteration of numerous intralobular branches (Figs 4 and 5 *a*) This process of obliteration was frequently accompanied by a vascular and perivascular lymphocytic infiltration In many places there was deposition of anthracotic pigment around obliterated pulmonary artery branches (Fig 4 *b*)

The segmental character of these obliterating lesions should be stressed Distal to an arterial segment with fibrous obliteration or a plexiform lesion the arterioles were dilated and thin walled These dilated vein like pulmonary arteries emptied into precapillaries and alveolar capillaries without obliterative features In fact the alveolar capillary bed appeared overdistended in some areas possibly in relation to intralobular collateral circulation

In places contrast medium had reached the dilated arteriole and the alveolar capillary bed *distal* to an obliterated segment of the pulmonary artery This could be demonstrated in microangiograms as well as in the serial sections and the following alternative collateral pathways to the alveolar capillary bed were recognized

(*a*) pleural and septal arteries (some of which

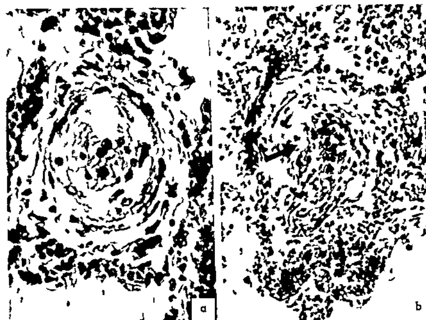


Fig 5 Pulmonary vascular lesions case 2 (*a*) Small, but thick walled muscular pulmonary artery with obliterated lumen and prominent mural necrosis Slight perivascular lymphocytic infiltration Hematoxylin eosin ×430 (*b*) Plexiform lesion with focal fibrinoid necrosis (arrow) There is moderate perivascular lymphocytic infiltration Hematoxylin-eosin ×170

possibly systemic) entering the pulmonary parenchyma to ramify as alveolar capillaries

(*b*) capillary communications with adjacent areas supplied by patent pulmonary artery branches and

(*c*) overdistended bronchiolar precapillary vessels

An area with collateral arterial supply of the alveolar capillary bed is shown in Fig 3

The bronchial arteries and the pleural systemic arteries showed normal histologic features for the age with a varying amount of longitudinal intimal smooth muscle bundles leading to complete obliteration of some branches This type of mural structure was also present in the two above mentioned arterial bronchopulmonary anastomoses of end to end type The intrapulmonary end to side anastomosis however showed no obliterative features The histologic appearance of the pulmonary and bronchial venous systems was normal

COMMENT

The preponderance of IPH in young females its familial incidence and its rapidly fatal course frequently with a duration of life from onset of symptoms of only 2 years or less (14) are all well known features of IPH and apply to the present two cases

Clinically the diagnosis IPH must be established by exclusion of any apparent causative lesion e g left to-right shunt intrinsic pulmonary disease or obstruction distal to the lungs In the present two cases neither cardiac catheterization or autopsy revealed such underlying mechanisms On the other hand the structural changes observed at autopsy probably played a significant role in maintaining the hypertension in the later stages of the disease as pointed out by Coleman et al (5)

The occurrence of dizziness and syncope after exertion is a common finding in IPH (28) Howarth and Lowe (15) have presented evidence that this may be due to acute failure of the right heart with a critical reduction in cardiac output. It is evident from Table I that the exercise cardiac output in case 2 was considerably lower than normal When exercise is completed under these conditions and the leg muscle pump no longer acts as a booster to the cardiac pump (17) the blood supply to the brain may become inadequate due partly to persisting vascular dilatation and blood pooling in the working muscles That the arterial oxygen saturation often shows a reduction with exercise in IPH (28) as also observed in case 2 (Table I) probably contributes to an insufficient oxygen transport to the brain

The low arterial oxygen saturation observed

during exercise in case 2 can be attributed to the significantly reduced oxygen saturation of the mixed venous blood (cf Table II) in combination with a direct right-to-left shunt flow through precapillary systemic pulmonary anastomoses Supporting evidence for the occurrence of significant true anatomical shunts in IPH has been obtained from the pure O_2 technique by Goff and Gaensler (9) In the present study some right to-left flow may well have occurred through the arterial bronchopulmonary anastomoses demonstrated at the histologic examination in case 1 (see Results)

The finding that in case 1 the infusion of a vasodilator drug reduced the pulmonary vascular resistance considerably ten months before her death and only slightly four months later agrees well with earlier reports on the effects of vasodilator drugs in IPH (for review see (19)) These indicate that vasoconstriction plays a subsidiary role in the pressure elevation in the earlier stages of the disease, but not later on, when organic changes become more extensive

The presence of probably irreversible obliterative lesions in the pulmonary arterial system was confirmed in both cases by angiographic and histologic techniques. These vascular lesions are [illegible]milar to those in previously reported cases of [illegible] (31) Survey post mortem angiograms from [illegible] 1 had the characteristic "pruned" appearance [illegible]esembling "the denuded shrub in winter" (6) and widespread narrowing and obliteration of the intralobular arterial bed was obvious in the microangiograms.

Peripheral to the obliterated segments of the pulmonary arteries there was a normal or in places, dilated alveolar capillary bed. This fits in with the observation by McCredie (18) that the pulmonary capillary blood volume as estimated by the $D_{1\,CO}$ technique may be maintained in IPH Our study also confirms the suggestions of some previous investigators (11 23) that, in the presence of hypertensive obliterating arterial lesions, the intact alveolar capillary bed is to some extent supplied by intrapulmonary and pleural collateral pathways.

The patent arterial bronchopulmonary anastomoses that were demonstrated by microangiography and serial sectioning in case 1 further reflect the increased collateral arterial supply of the pulmonary parenchyma. Such anastomoses are normally present in the fetal and infant lung (24 33) but not in the adult lung (27) The anastomoses in case 1 however seem to be few in number and our study fails to support the theory of abnormal arterial bronchopulmonary anastomoses as the primary cause of IPH (2 4 7 34)

The possible role of occult pulmonary thromboembolism in the pathogenesis of IPH has been considered by some authors (3 12 16) In one of our cases an organized embolus was found in a lobar branch of the pulmonary artery However single organized pulmonary emboli of this size do not seem to be able to initiate persistent pulmonary hypertension Furthermore since the mural changes in the peripheral pulmonary arteries of our two cases suggest gradual cellular intimal proliferation with eventual fibrous obliteration, we do not believe that chronic pulmonary thromboembolism was the cause of IPH in these two siblings

The present report includes the first angiographic documentation of the plexiform lesions a highly characteristic feature of severe pulmonary hypertension. Previous studies on the structure of the plexiform lesions with serial sectioning have shown that this lesion is entirely arterial and that its efferent vessels terminate as alveolar capillaries (1 21 22, 29) Our microangiographic findings confirm these earlier observations Some of the plexiform lesions demonstrated in case 1 allowed the transmission of contrast into dilated arterioles (Fig. 2) which in the serial sections could be traced down to alveolar precapillaries and capillaries

Although vasoconstriction may have contributed to the increased medial thickness of the muscular pulmonary arteries our morphometric results strongly suggest true medial hypertrophy This is particularly true for case 2 in which the pulmonary arterial system was injected and hence to some extent distended by contrast medium The medial index of this case (10%) was nevertheless twice the normal. Medial index values above 10% are ordinarily not encountered after the age of one month (32) It cannot be determined from our study however whether the medial hypertrophy is secondary to the hypertensive state or whether there is some primary carry-over of the fetal medial structure in the muscular pulmonary arteries as has been suggested in cases of IPH occurring in infancy (10 13 20 25)

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Congress Announcements

The Third International Congress of Social and Preventive Medicine will be held in Lido of Venice Italy May 24 to 28 1970

Information Segreteria del III° Congresso Internazionale di Medicina Preventiva e Sociale Ospedale al Mare 30126 Lido di Venezia Italy

The Second Czechoslovak Congress of Internal Medicine with International Participation on the Topic of *Pathogenesis and Therapy in Edema* will be held in Bratislava September 15 to 18 1970

Information Congress Office First Clinic for Internal Diseases Mickiewiczova 13 Bratislava Czechoslovakia

The Second Congress of the European Association of Radiology will be held in the International Congress Centre RAI in Amsterdam June 14 to 18 1971

Information The Congress Secretariat c/o Holland Organizing Centre 16 Lange Voorhout The Hague Holland

SUBJECT INDEX

(Supplements see page XII)

Diabetes mellitus

Endocrine glands

Hypertension

Infection

Kidney

Liver

Lung

Metabolism

Muscles

Population studies

Rheumatic diseases

Skin

Treatment

Tumours

Urinary tract

Veins

SUPPLEMENTS TO VOLUME 186

499 Studies on the pathogenesis of post traumatic fat embolism

- I *The chemical composition of the fat emboli in the post-absorptive dog* By J Kerstell B Hallgren C M Rudenstam & A Svanborg
- II *Serum lipoprotein pattern in fat embolism in the dog* By A Gustafson & J Kerstell
- III *The chemical composition of the fat emboli in the post-alimentary dog* By B Hallgren J Kerstell C M Rudenstam & A Svanborg
- IV *The incorporation of labelled chylomicrons into the fat emboli in the post-alimentary dog* By J Kerstell
- V *The chemical composition of the fat emboli in dogs with extirpated liver and intestines* By J Kerstell
- VI *The influence of increased and decreased plasma free fatty acids on the formation and composition of the fat emboli in dog* By B Hallgren J Kerstell C M Rudenstam & A Svanborg

500 Glucocorticoids in internal medicine A symposium on systematic therapy other than substitution therapy Redigenda curavit Ib Lorenzen

The physiology and pharmacology of the glucocorticoids By Christian Binder
The effects of the glucocorticoids on connective tissue By Ib Lorenzen
Glucocorticoids in rheumatic fever and rheumatoid arthritis By Eigill Hvidberg
Glucocorticoids in the connective tissue diseases By Ib Lorenzen
Glucocorticoids in haematology By Aage Videbæk
Glucocorticoids in allergic diseases By Michael Schwartz
Glucocorticoids in liver diseases By Flemming Quaade
Glucocorticoids in gastro-enterology By Povl Riis
Glucocorticoids in nephrology By Herluf Jensen
Glucocorticoids and infectious diseases By Poul Elfersoe
The function of the pituitary gland and the adrenal cortex during treatment with glucocorticoids By Christian Binder
Undesirable effects of glucocorticoids By Flemming Quaade
Concluding remarks By Ib Lorenzen

501 Throat streptococci in rheumatoid arthritis By Auli Toivanen

502 Long term cardiac pacing Experience of fixed rate pacing with an endocardial electrode in 260 patients By Olof Edhag

503 The frequency clinical picture and prognosis of pulmonary sarcoidosis in Finland By Olof Selroos

LIST OF AUTHORS